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AN ENZYME APPROACH TO THE PREVENTION AND REMOVAL OF GELATINOUS FILMS IN RAW SEWAGE ULTRAFILTRATION SYSTEMS

DAVID W. TAYLOR NAVAL SHIP RESEARCH AND DEVELOPMENT CENTER

Bethesda, Maryland 20084

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AN ENZYME APPROACH TO THE PREVENTION AND REMOVAL OF GELATINOUS FILMS IN RAW SEWAGE ULTRAFILTRATION SYSTEMS

Prepared Under Contract N00167-77-C-0001 by
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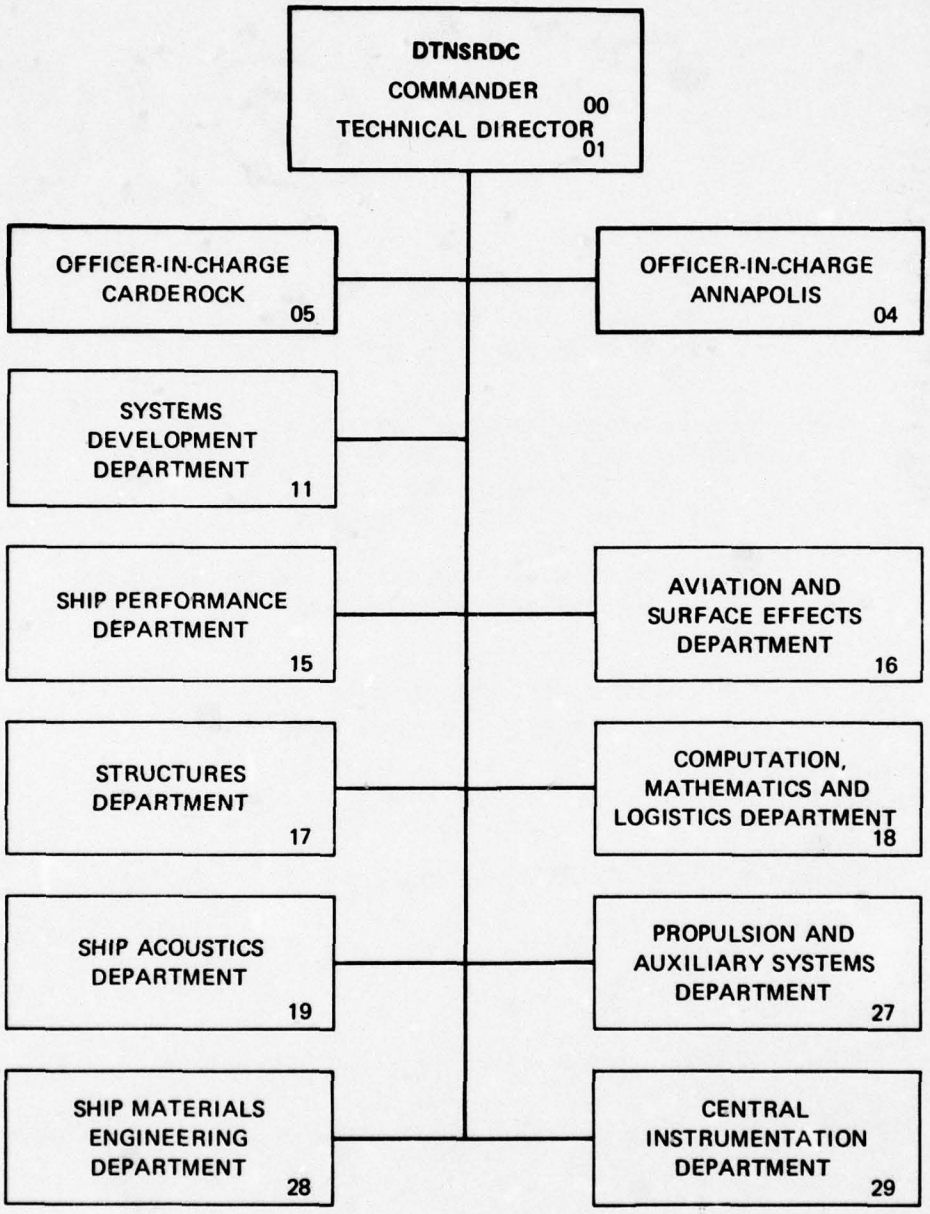

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Both the Navy and the Army have investigated shipboard wastewater treatment systems to meet marine discharge regulations. Ultrafiltration membrane technology is one method that has been evaluated. However, membrane fouling due to concentration polarization at the membrane surface affects ultrafiltration performance and accounts for the decline of the effluent (permeate) rate with time. Numerous techniques have been reported in the literature for

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overcoming this problem, but the potential solutions have been of a temporary nature, requiring frequent cleaning to offset the decline. In-situ removal of the fouling layer would greatly enhance ultrafiltration's effectiveness.

A concept for overcoming the fouling problem, which was not previously reported, has been investigated by Rutgers University. It involves immobilizing enzymes onto ultrafiltration membranes. It was speculated that enzyme action would degrade the fouling layer as it was formed.

The fouling layer produced on ultrafiltration membranes in processing raw sewage has been determined to consist of mostly proteins with a smaller amount of lipids. Candidate enzymes were evaluated for their ability to degrade this fouling layer. One enzyme, a protease, was selected for immobilization on the membranes. Immobilization was by vacuum adsorption.

Because of sewage's complex composition, evaluation of an immobilized-enzyme-membrane's (IEM) performance showed considerable variation. A homogeneous fluid, consisting of a 0.1 percent nonfat dry milk solution was thus selected as a model to demonstrate the enzyme concept. IEM performance processing this milk protein solution was established. One 240-hour test showed the IEM produced more than a 6-fold increase in effluent relative to a non-IEM control. Shorter term tests showed similar results. Enhancement of permeate rates and, consequently, increased permeate production appear to be directly related to the immobilized enzyme action. Evaluation of a mixed IEM system (protease, lipase, cellulase, etc.) was recommended for future evaluation with raw sewage.

PREFACE

This contract was funded by the David W. Taylor Naval Ship R&D Center (DTNSRDC), Annapolis, Maryland, and the US Army Mobility Equipment R&D Command (MERADCOM), Fort Belvoir, Virginia. Mr. Lynne Harris, DTNSRDC, was the principal contract monitor and Mr. Dan Lent, MERADCOM, was the co-monitor. Both the Navy and the Army have investigated ultrafiltration as a means of treating shipboard sewage. Several reports have documented the feasibility of using ultrafiltration for this application (Harris and Adema, Hoover, et al). However, membrane fouling has been identified as one of the possible limitations.

This contract was undertaken to study the effect of enzymes on minimizing the membrane-fouling problem. Because of the complexity of sewage compositions and variability of sampling, inconsistent results were obtained. In order to assess the true effect of enzymes, it became necessary to use a model system instead of the actual waste water. The results of experiments are included in this final report and interim reports dated March 1977 and December 1976 (Appendices B and C).

SUMMARY

Rhozyme P-53, a commercial food grade protease, was immobilized on an Abcor HFM membrane, an ultrafiltration membrane with a 20,000-molecular weight cutoff. Immobilization is effected by a "vacuum" technique. The process involves, first, creating a vacuum in the tubular membrane module (1 in. ID, 5 ft long) followed by the introduction of an enzyme preparation into the module through metered release of the vacuum. The drawn-in enzyme solution is allowed to stay in the module for 24 hours.

A prototype unit, consisting of Rhozyme P-53 immobilized on an Abcor HFM membrane in a tubular form, was used to ultrafilter 0.1 percent nonfat dry milk solution. Permeate and retentate streams were returned to the holding tank to achieve a complete recycle operation. At an operating temperature of 50° C and a retentate recirculation rate of 30 gal/min (corresponding to 40 lb/in^{2}g inlet pressure in the system), the prototype unit was found to have a half-life six times that of the control based on permeate flux data. The flux of the control dropped to 16 gal/ft^2/day in 42 hours of continuous filtration time, whereas the prototype flux reached the same level after 240 continuous hours. Using 16 gal/ft^2/day as a cutoff point, the prototype unit produced a total permeate of 260 gallons. By contrast, the control produced only a total of 34 gallons. In other words, the prototype unit produced a 664 percent increase in total permeate relative to the control. The effect of the immobilized enzyme on the

bulk phase hydrolysis of proteins was negligible in the quasi-steady-state regime of the flux/time curve. The mechanism for flux enhancement was found to be unquestionably linked to the enzyme action on the gelatinous layer at the membrane wall interface.

List of Abbreviations

in	- inches
ID	- inside diameter
°C	- degrees centigrade
gal/min	- gallon per minute
lb/in^2g	- pound per square inch gage
$gal/ft^2/day$ (GFD)	- gallon per square foot per day
%	- percentage
w/v	- weight per volume
r/min	- revolution per minute
NFDM	- nonfat dry milk
O.D.	- optical density
TCA	- trichloroacetic acid
ml	- millilitre
nm	- nanometre
V_{max}	- maximum velocity
pH	- negative logarithm of the hydrogen ion concentration
UF	- ultrafiltration
M	- molarity
HCL	- hydrochloric acid
w/w	- weight per weight
IEM	- immobilized enzyme membrane
H_2O	- water
mg/l	- milligram per litre
g	- gram
cm^2	- square centimetre

TABLE OF CONTENTS

LIST OF FIGURES

AN ENZYME APPROACH TO THE PREVENTION AND REMOVAL OF GELATINOUS FILMS IN RAW-SEWAGE ULTRAFILTRATION SYSTEMS

INTRODUCTION

During the last decennium, ultrafiltration has been added to the repertoire of engineers engaging in separation processes. However, its applications are not expanding as fast as expected. The main technical difficulty involved in using the process is often referred to as a phenomenon called concentration polarization, gelatinous film-buildup, or membrane fouling. The result of such a phenomenon is a reduction in membrane throughout (flux).

Essentially, this phenomenon is due to the deposition of solutes on the surface of the membrane. The deposition exerts its effect on membrane flux in two ways - directly as a hydraulic resistance, and indirectly via the increased concentration polarization in the stagnant boundary layer caused by the deposits.

Leiserson (1973) reported that fouling of a membrane can be caused by heavy metal oxides, bacterial slimes, calcium sulfate, calcium carbonate, organic colloids, and inorganic colloids. Generally speaking, several types of compounds are encountered together, and a synergistic effect between these compounds is highly possible. "Organic material" is a category that is most frequently reported as the responsible deposit in fouling of the membrane by researchers in this field. Lim et al (1971) reported high content of casein deposits in ultrafiltration of whey. Cruver and Nusbaum (1974) found mainly polyhydroxy aromatics in deposits from waste water treatment. Beckman et al (1973) found 26% organic acids and 44% polysaccharides in the deposits from the ultrafiltration of a polluted surface water sample.

One of the ways to decrease the deposition of such organic materials is membrane modification. Fouling has been attributed to the existence of negatively charged colloids in the feed. Therefore, negatively charged membranes, a cellulose acetate-hydrogen succinate membrane (Fisher and Lowell, 1970), and some highly sulfonated membranes (Gregor, 1973) were developed.

Another approach to the modification of the membrane is the immobization of enzymes to the membrane, thus creating a self-cleaning mechanism on the membrane surface. Fisher and Lowell (1970) attached trypsin to a reverse-osmosis membrane cast from cellulose acetate N-hydroxy-succinimide. The membrane showed enzymatic activity toward BAEE, a synthetic substrate, but no actual tests with the membrane were reported. Dejmek (1972) bound trypsin to cellulose acetate through a cyanogen bromide coupling method. Although he found proteolytic activity of the immobilized trypsin, the membrane did not behave differently from controls in runs with proteinaceous solutions.

Attachment of enzymes to ultrafiltration membranes through covalent bonds which require proper activation (such as by cyanogen bromide, triazine, and other nucleophilic agents) can be expected to modify the intrinsic permeation properties of the membrane. With the advent of immobilized enzyme technology in the early 1970's, there are many different methods of attaching enzymes to their carriers. Immobilization of enzymes through multiple secondary bonds (Van der Waals bonds, electrostatic bonds. hydrophobic bonds, and hydrogen bonds) requires a much less severe reaction environment than creating a covalent bond. This is obvious from an energetic point of view. Secondary bonds are easily formed between protein-protein interactions (Wang and Vieth, 1973), between an enzyme and an ion exchanger,

and between an enzyme and an adsorber such as alumina. Using such multiple-bonds-formation techniques, one probably would be able to attach enzymes to ultrafiltration membranes without modifying the intrinsic permeation properties of the membrane.

In an attempt to test the hypothesis of enzymatic control of gelatinous films developed during ultrafiltration of raw sewage, we have selected a model analog system consisting of an immobilized protease-ultrafiltration membrane for the processing of nonfat dry milk solutions. The fouling of the test membrane during ultrafiltration of raw sewage is at least partially due to the deposition of proteinaceous materials. The method of immobilization used in this study is that of adsorption which involves the formation of secondary bonds.

EXPERIMENTAL

APPARATUS - A sketch of the continuous recycle apparatus (prototype unit) is shown in Figure 1. Protein solutions are made up in a stainless steel holding tank (11).* During an experiment, the solution passes across the membrane (6) surface under pressure producing a permeate stream which is collected through a tube connected at the lower section of the membrane cartridge (7) and a retentate stream which is returned to the tank through the valve (10). A bypass section (12) is also included so that solutions may be returned to the tank without passing through the membrane.

During the immobilization procedure, a vacuum pump is attached to the line below the valve (9) while all immediate inlets to the membrane (4, 5, 7, and 10) are closed. When a sufficient vacuum has been created, the valve (9) is closed and the enzyme solution is drawn in through the inlet (5).

THE MEMBRANE AND THE ENZYME. The membrane used in this study is an organic polymeric membrane, type HFM from Abcor, Wilmington, Massachusetts. Test pairs of these membranes in tubular form (5 feet long, 1 inch I.D.) were purchased for experiments. The molecular weight cutoff of these membranes is 20,000.

The enzyme used in the studies is a bacterial protease, Rhozyme from Rohm and Haas Company, Philadelphia, Pennsylvania. Two grades of the Rhozyme, B-6 and P-53, were used.

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*Numbers in parentheses refer to corresponding numbers in Figure 1.

IMMOBILIZATION PROCEDURE. Approximately 1½ liters of a 10% (w/v) solution of Rhozyme P-53 (Rohm and Haas Co.) are prepared and centrifuged at 8000 r/min for 20 minutes. The supernatant is collected and adjusted to a pH equal to 5.1 with dilute hydrochloric acid. The Abcor HFM membrane tube is subjected to a vacuum of 25.6 inches of mercury for 1 hour. When the vacuum in the membrane is sufficient, the enzyme solution is drawn into the membrane chamber and onto the membrane surface. The membrane is allowed to soak in enzyme solution for 24 hours, and then the excess enzyme solution is drained off.

To determine the steady-state water flux, water is circulated through the system and the flux is measured at 5-minute intervals until the flux reaches a constant level. The steady-state water flux, which is usually attained within 30 minutes, is recorded. Then the system is flushed with 20 gallons of fresh water and is drained completely. This process is repeated at least three times. After this treatment, it can be assumed that all loosely bound enzyme has leached out and been removed from the system. The prototype is ready for use at this time.

EXPERIMENTAL PROCEDURE. The tank is initially filled with 20 gallons of 0.1% (w/v) nonfat dry milk (NFDM) protein solution. The fluid is circulated at a volumetric flow-rate of 30 gallons per minute at a pressure drop across the membrane of 40 lb/in^2. The temperature is maintained at 50° C by means of a stainless steel heat exchanger unit through which hot tap water is passed and controlled manually. Since both the circulating fluid and permeate are returned to the tank, the volume of the tank remains essentially constant.

Permeation fluxes are noted at frequent intervals since these data form a reliable basis by which the success of a particular experiment may be judged. Permeate and retentate samples are collected at less frequent intervals to be analyzed by the Lowry Titration Method (Lowry, 1951).

When nonfat dry milk solution is used with an immobilized enzyme membrane (IEM), retentate samples are collected frequently in the beginning after fresh solution is added. These samples are used to follow protein hydrolysis by two methods; O.D. measurement at both visible and ultraviolet wavelengths and milk precipitation by TCA (Trichloroacetic acid) method. When raw sewage is used, a retentate sample is collected to determine the actual solids content in the tank.

Usually, the system is stopped at 10-hour intervals and the spent solution is drained. Fresh solution is then added and the system started up immediately. When an entire experiment is completed, the system is cleaned. All results are shown in Appendix A.

MEMBRANE CLEANING PROCEDURE. At the termination of an experiment, the tank and piping are emptied. The system is filled with water, flushed, and drained again. This procedure is repeated until the tank is free of all particulate matter.

A commercial-grade Chlorox solution (25 ml) is added to 20 gallons of water and circulated through the system with the permeate line closed and a 0 lb/in^2 net pressure drop across the membrane. Any gel material or sedimented material which coats the surface of the membrane will be stripped off the surface. At the end of this phase of the cleaning process, the system is drained and refilled with 20 gallons of fresh water and 25 ml of Chlorox. This time, the system is flushed with the permeate line open and a pressure drop across the membrane equal to approximately 40 lb/in^2g. In this way, the pores of the membrane are cleansed.

ESSENTIAL IMPROVEMENTS OF THE SYSTEM DEVELOPED SINCE MARCH 1977

Many significant improvements have been incorporated into the experimental procedure used in earlier research (refer to Appendices B and C). All of these improvements have contributed to the dramatic increase in permeate flux exhibited by the immobilized enzyme ultrafiltration system as compared to the control. These changes include the substitution of the enzyme Rhozyme P-53 (a more concentrated enzyme) for Rhozyme B-6 (an enzyme preparation that is diluted with wood flour); increased efficiency of enzyme adsorption during the immobilization procedure; operating the system under more optimal process conditions; and improving the membrane cleaning procedure between experiments.

SUBSTITUTION OF RHOZYME P-53 FOR RHOZYME B-6. Rhozyme P-53 (Rohm and Haas Co.) has recently been substituted for the previously used enzyme Rhozyme B-6. Rhozyme P-53 is essentially a more concentrated and potent form of Rhozyme B-6. Rhozyme B-6 has insoluble microbial cells and wood flour dilutants which must be separated by centrifugation to prevent membrane pore clogging. Rhozyme P-53 has a much lower proportion of these dilutant materials.

Considering its higher activity and increase in soluble fraction, as compared to Rhozyme B-6, Rhozyme P-53 is actually more economical than Rhozyme B-6. Recycling the unused portion of the enzyme solution directly after immobilization procedure and/or using lower enzyme concentrations

have not been studied. These economical measures may further reduce the enzyme cost.

Rhozyme P-53 has all the advantages of Rhozyme B-6 (specific applicability to the ultrafiltration system used in these experiments). The former enzyme is a potent protease and would be most effective in breaking down the gel layer formed during the ultrafiltration of sewage, since protein is suspected to be the largest component in this gel layer. The optimum activity of Rhozyme P-53 occurs within a pH range of 5 to 8.5 with the maximum activity between pH 6 and pH 7. Such pH ranges correspond to the pH's of both sewage and nonfat dry milk. Rhozyme P-53 is also extremely active at the operating temperature of 50° C. Finally, it is simple to immobilize Rhozyme P-53 by the vacuum adsorption technique developed in the course of this research.

INCREASING THE EFFICIENCY OF ENZYME ADSORPTION DURING IMMOBILIZATION. It is believed that the membrane is negatively charged; hence, a new step has been introduced into the immobilization process to bind the enzymes more strongly to the membrane by making their net charge positive. This is accomplished by adjusting the pH of the enzyme solution to a pH below the isoelectric pH of the enzyme solution. (Lehninger, 1970.)

Experimentally, by means of an acid-base titration, the isoelectric point of a 10% (v/w) Rhozyme P-53 solution was determined. The isoelectric pH is found to be 5.5 (see Figures 2 and 3). Since the enzyme is active in an optimal pH range between 5 and 8, it is possible to make the enzyme solution positively charged by lowering the pH of the solution to 5.1 without interfering with enzymatic activity.

It should be noted that although immobilization of the enzyme is conducted at a lower pH, the actual operation of the enzyme reactor is performed at the optimum pH of the enzyme (pH 7).

INCREASING THE OPERATING TEMPERATURE FROM 35°C TO 50° C. One major improvement in the present system over the earlier system is that the operation is at 50° C rather than 35° C. Increasing the temperature to this level does no damage to the membrane itself and has been largely responsible for the success of the Rhozyme P-53 immobilized system.

First, 50° C is much closer to the optimum temperature of Rhozyme P-53 than 35° C. Rohm and Haas report that the activity approximately doubles for each 10° C increase between 40° C and 60° C, after which the activity falls off rapidly. The enzymatic activity at 50° C is more than twice that at 35° C, and maintenance of the system at 50° C safely avoids the danger of exceeding the critical temperature of approximately 60° C for the membrane. It may be assumed that this increase in enzymatic activity accounts for much of the relative flux improvement observed in the latest prototype experiments.

Furthermore, at 50° C, microbial growth is minimized. During an immobilized enzyme membrane experiment at 35° C, severe drops in flux, accompanied by significant drops in pH due to microbial growth, was observed after about 30 hours of operation. Once serious microbial growth had been observed, it recurred within hours in spite of frequent replenishments of fresh substate. During a prototype run at 50° C, however, no serious bacterial growth was observed until after 100 hours of operation.

The tank was cleaned, and bacterial growth was not observed until another 80 hours had elapsed.

Since 50^{o} C closely approximates the steady-state temperature of the system (equilibrium between radiation losses and heat gained by the pump action and fluid friction), maintenance of the system at this temperature is easy. All that is required is a simple stainless steel coil through which hot tap water may be passed when needed. It is still essential that the system be maintained at a constant temperature to avoid variations in flux and to simplify mathematical modeling. It should be noted, however, that an increase in temperature will cause some increase in flux for both the control and prototype.

TOTAL RECYCLING AND MAINTENANCE OF CONSTANT VOLUME AND MASS. In the most recent studies, the system has been changed from a partial recycle to a total recycle system by returning the premeate to the tank rather than discarding it. This system is then essentially one of constant volume (with small amounts of water added to account for evaporation losses) and constant mass. This change will greatly simplify the task of mathematical modeling of the system in the future.

MEMBRANE CLEANING PROCEDURE. An addition to the membrane cleaning procedure between experiments allows better regeneration of the original membrane properties (as indicated by steady-state water fluxes). This improvement lies in the ability to "strip off" both the gel layer and immobilized enzymes from the surface of the membrane.

The stripping-off process is accomplished by flushing 20 gallons of water and 25 milliliters of Chlorox solution through the system with the permeate line closed and a 0 lb/in^2 pressure drop across the membrane.

Essentially, no solution is forced through the membrane, so the gel layer and/or immobilized enzymes are stripped off the membrane by the shear from the circulating fluid without normal forces due to flow through the membrane.

Less Chlorox is used which decreases the chances of membrane damage and makes it easier to remove all traces of Chlorox. This is an advantage because any Chlorox remaining on the membrane might poison the enzyme molecules to be immobilized on the same membrane in later experiments.

After membrane cleaning, no difficulties are experienced when fresh enzyme is reimmobilized on the membrane and additional experiments are performed. This indicates that the cleaning process has not altered the mechanical or physical properties of the membrane significantly. Thus, the half-life of the membrane is extended considerably since it can be reused after the improved cleaning procedure.

EXPERIMENTAL RESULTS FOR THE CONTINUOUS RECYCLE ULTRAFILTRATION OF NONFAT DRY MILK

COMPARISON OF PROTOTYPE PERFORMANCE TO CONTROL

FLUX. Figure 4 shows a comparison of the observed flux for the ultrafiltration system without the immobilized enzyme (referred to as the "control") and for the immobilized enzyme (called the "prototype"). Both the prototype and control experiments were continued until both flux levels reached 16 GFD. This flux was chosen as the lower limit of operation. The superiority of the prototype to the control is clearly indicated by the fact that the control flux dropped to 16 GFD in only 42 hours, whereas the prototype flux reached the same level after operation for 240 hours. In terms of total volume of permeate collected until the time at which the permeate flux drops to the lower limit flux (16 GFD), the prototype produces 260 gallons, whereas the control produces only 34 gallons. The prototype system is, therefore, approximately 664% better than the control based on those figures ($\frac{259 - 34}{34} \times 100 = 664\%$).

Assuming that the control maintains a flux of 12 GFD from 50 to 240 hours (when, in fact, the flux will decrease with time), the average flux is 13 GFD, and 134 gallons of permeate will be produced during a 240-hour period. In the same time span, the prototype will produce 259 gallons of permeate and have an average flux of 26 GFD.

Over a 150-hour period, the prototype produces 187 gallons of permeate (average flux is 30.0 GFD) and the control produces 89 gallons

(average flux is 14 GFD). For a 50-hour period, the prototype produces 84.0 gallons of permeate (average flux is 40 GFD) and the control produces 39 gallons (average flux is 18.5 GFD).

In terms of average fluxes, it may be noted that the prototype shows a consistent improvement of about 100% for the entire 240-hour period of operation.

Several interesting facets of the curve in Figure 4 may be noted. During the first 10 hours of the prototype experiment, a high degree of hydrolysis of milk protein occured in spite of the removal of all loosely bound enzyme prior to the experiment. This corroborates the high activity of Rhozyme P-53 and its applicability to the ultrafiltration system used in this research, as compared to Rhozyme B-6.

When fresh milk solution is added after the first 10 hours of prototype operation, the flux drops rapidly within 2 hours to the corresponding control flux (i.e., about 21 GFD), but rises again to 42 GFD. This phenomenon probably corresponds to a transition period in the process. It is postulated that at a quasi-steady-state, which is reached after the transition period, an equilibrium is reached between enzymatic hydrolysis of the gel layer and the building up of the gel layer.

The gradual decay in permeate flux with time might be attributed to the gradual deactivation of the immobilized enzyme. As the enzyme loses its activity, the gel layer thickness probably increases and so decreases the permeability of the membrane by creating an additional resistance. The immobilized enzyme remained active long enough to maintain the flux at levels much higher than the control for 240 hours. The overall gross

slopes of the two curves in Figure 4 are nearly parallel straight lines. This observation may, perhaps, offer insight into the mechanism of flux enhancement. Consideration of factors like this will be taken up in future work.

In conclusion, the immobilization of Rhozyme P-53 dramatically improves membrane flux in the ultrafiltration process used in this research. Maintenance of desired flux levels was achieved for a much longer period (more than 200 hours) with the immobilized enzyme than without it. Specifically, the half-life of the prototype is about six times longer than that of the control. The volume of permeate produced by the prototype was about 664% more than that produced by the control.

PROTEIN CONTENT IN THE RETENTATE AND PERMEATE. From Figures 5 and 6, it is evident that the protein concentration in the retentate remained constant. This is to be expected since the system is designed to be a constant mass system. Variations at 10-hour intervals may be attributed to the preparation of fresh solutions at these times.

The time history of the protein concentration in the tank is more complex. The control system experienced a sharp increase in protein concentration during the first hour, followed by a drop to a low level which was maintained for the rest of the time it was measured. The higher level of protein in the permeate during the first hour was probably due to the better membrane permeability before the gel layer build up.

In the permeate of the prototype, protein content reached high levels during the first two 10-hour intervals. When the milk solution was hydrolyzed, in addition to the gel layer, much more protein was able to permeate

the membrane. However, once an equilibrium was established between the gel layer build up on the membrane and enzymatic hydrolyses of the gel layer (after 25 hours of operation), the protein content in the permeate dropped to levels similar to those in the control.

MEASUREMENT OF DEGREE OF PROTEIN HYDROLYSIS. Considerable research effort was expended to devise methods of following protein hydrolysis in the system. If this could be achieved, it might be possible to propose mathematical models for the system which would relate membrane flux to the immobilized enzyme activity under actual operating conditions. The problems of studying smaller systems and adapting the results to the present unit could then be avoided.

BATCH STUDIES. In Figures 7 and 8, the enzymatic activity of free Rhozyme P-53 (all fractions and soluble fractions only) on 500 milliliters of 0.05% nonfat dry milk is studied in a batch reactor. The degree of hydrolysis is measured by recording the optical density at both visible (λ = 540 nm) and ultraviolet wavelengths (λ = 280 nm). The results of the methods are consistent and indicate that the degree of protein hydrolysis can be followed by either of these methods.

OPERATION STUDIES. The methods used to follow protein hydrolysis during a continuous recycle experiment show that enzymatic activity curves can be formed only when hydrolysis of the tank contents occur (during the first 10 hours of operation). This does not indicate a lack of immobilized activity after 10 hours of operation. It does indicate that a continual breakdown of the gel layer on the membrane surface produces small quantities of hydrolyzed protein which, when diluted in a volume of 20 gallons, can not

be detected by the methods used. The fact that continual enzymatic gel layer hydrolysis occurs is postulated by the membrane flux studies.

Fresh milk solutions are used to replace used solutions at regular intervals to provide an environment with zero product concentration. The maximum velocity (V_{max}) of the immobilized enzyme is obtained by measuring the slope of the activity curve at this point. By repeating this procedure at regular intervals, it was thought that with time the deactivation of the enzyme could be followed. Inspection of Figures 8, 10, 11, and 12 demonstrates, however, that protein hydrolysis can not be measured when only hydrolysis of the gel layer occurs.

In the continuous recycle experiments, the results of an attempt to follow protein hydrolysis by milk precipitation method using trichloroacetate solution are shown in Figure 9. This method is an adaptation of a standard method (Yasunobu and McConn, 1970) by which trichloroacetate solution is added to a retentate sample rather than to casein. As protein hydrolysis increases, the optical density measured at a wavelength of 280 nm increases. Figure 9 shows an increase in optical density at this wavelength as the tank contents are hydrolyzed during the first 10 hours of operation. When fresh solution is added after the initial 10 hours, hydrolysis is no longer measurable.

Figure 10 shows the changes in optical density at λ = 280 nm of the retentate with time. As with the free enzyme studies (Figure 7), the optical density decreases as hydrolysis increases. After fresh solution is added, the optical density remains high and constant, indicating the lack of measurable protein hydrolysis.

The change in optical density at λ = 540 nm is shown in Figure 11. Again, as with the free enzyme studies (Figure 7), the optical density decreases as protein hydrolysis increases. During the first 10 hours of operation, a good activity curve is produced. Later, the optical density readings become quite erratic and it may be assumed that no measurable hydrolysis occurs.

Another indication of protein hydrolysis is the increase of protein in the permeate. This is not as reliable as the other methods since membrane defects may also be responsible for increases of protein in the permeate. Figure 12, however, shows a valid correlation between protein hydrolysis and increased protein content in the permeate during the first 10 hours of operation.

COMPARISON OF THE PROTOTYPE USED PRESENTLY TO THE PROTOTYPE USED IN EARLIER EXPERIMENTS. Figure 13 shows the initial prototype tested for the present experiments. It is an immobilized Rhozyme P-53 system operated at 50° C with total recycle. The enzyme activity data in terms of protein hydrolysis measurements (shown in Figures 9, 10, and 11) were obtained during this experiment. The enzyme activity decreased more rapidly than the second prototype of this type (Figure 4) because the membrane was subjected to a washing process designed to strip off the enzyme. In spite of this treatment, the system still proved to be superior to the partial recycle, immobilized P-53 system operated at 35° C which was used in earlier experiments. Results are shown in Figure 14.

CONCLUSIONS AND RECOMMENDATIONS

In the ultrafiltration of nonfat dry milk solutions, this study showed that a protease immobilized on the membrane can significantly reduce membrane fouling problems. Based on flux data, the relative half-life of the prototype system with enzyme on the membrane is increased to six times that of the control. The volume of permeate produced by the prototype was about 664% more than that produced by the control. The overall gross slopes of the two flux versus time curves (Figure 4) are nearly parallel straight lines. Analysis of this observation may, perhaps, offer insight into the mechanism of membrane fouling and enhancement.

Ultrafiltration of raw sewage using a similar prototype with protease immobilized membrane showed erratic improvements of membrane control flux (Appendices B and C). The major component in milk is protein. Sewage is a more complex system than milk in terms of chemical and biochemical compositions. Despite this marked difference, one can still expect to extrapolate from the optimistic data of the nonfat dry milk system, that with proper selection of enzymes, results analogous to the milk experiments are possible. One enzyme system that promises to be effective and have an impact on flux performance is cellulase. Different cellulases have been produced commercially from fungal fermentation processes. We are now experimenting with different cellulases and with combined cellulase-protease immobilized membrane systems for the treatment of raw sewage.

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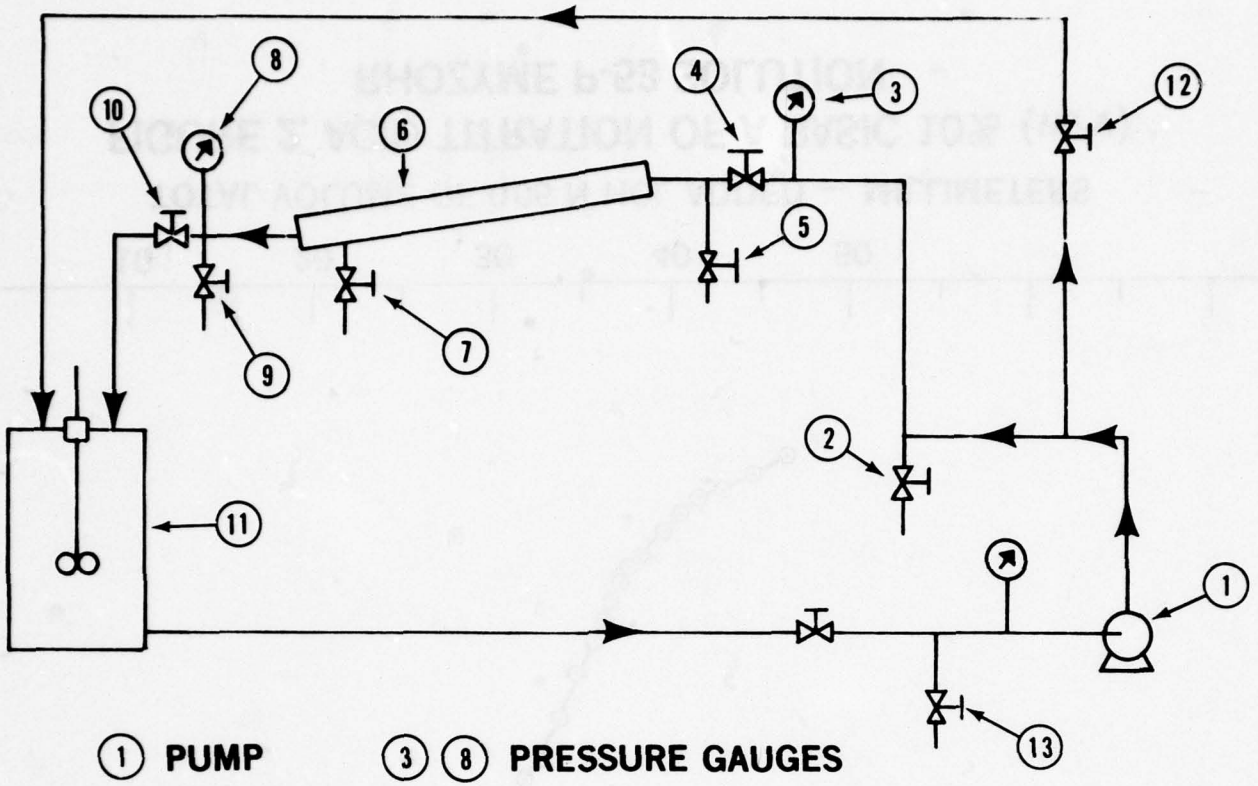

FIGURE 1. PROTOTYPE ULTRAFILTRATION UNIT

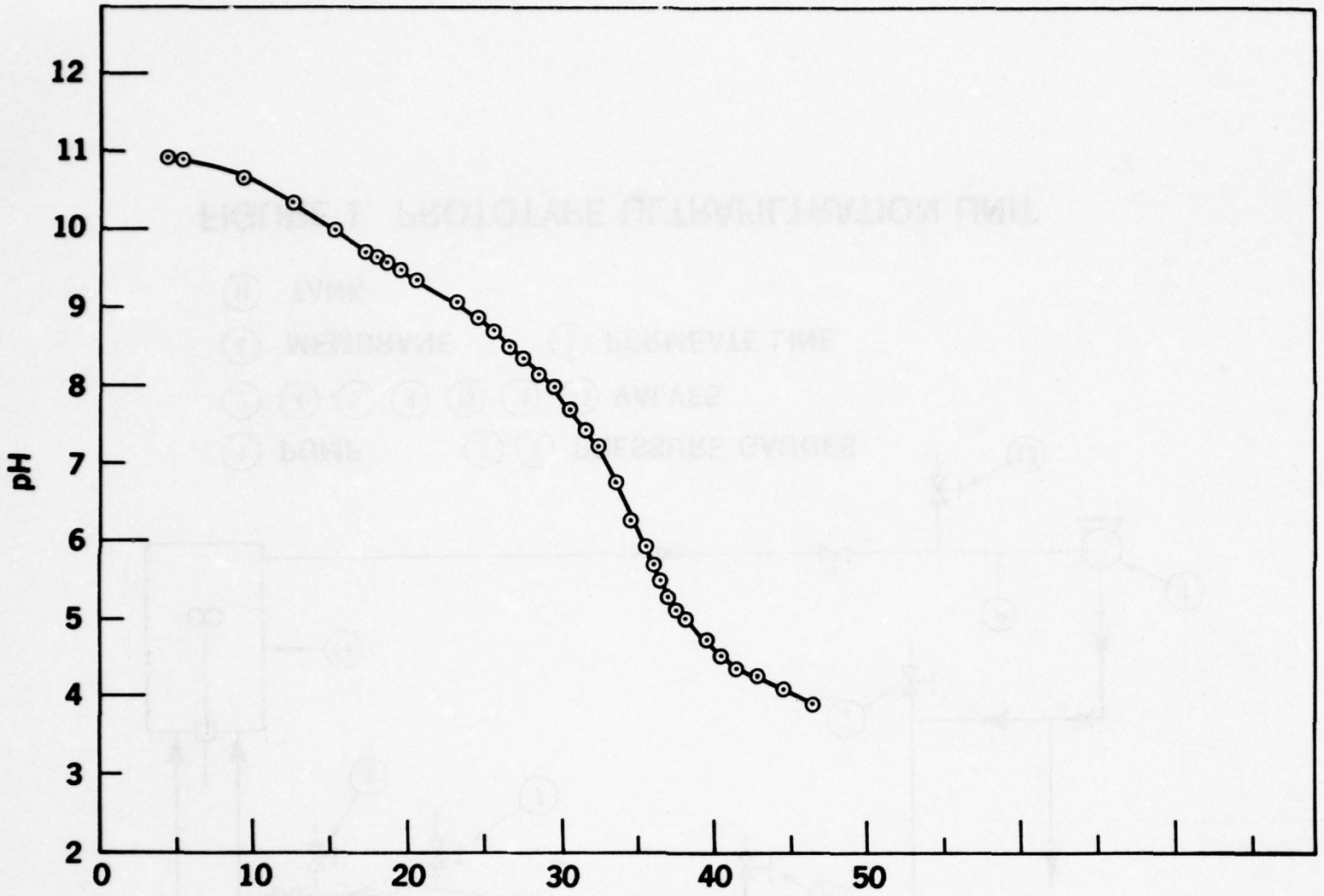

FIGURE 2. ACID TITRATION OF A BASIC 10% (w/v) RHOZYME P-53 SOLUTION

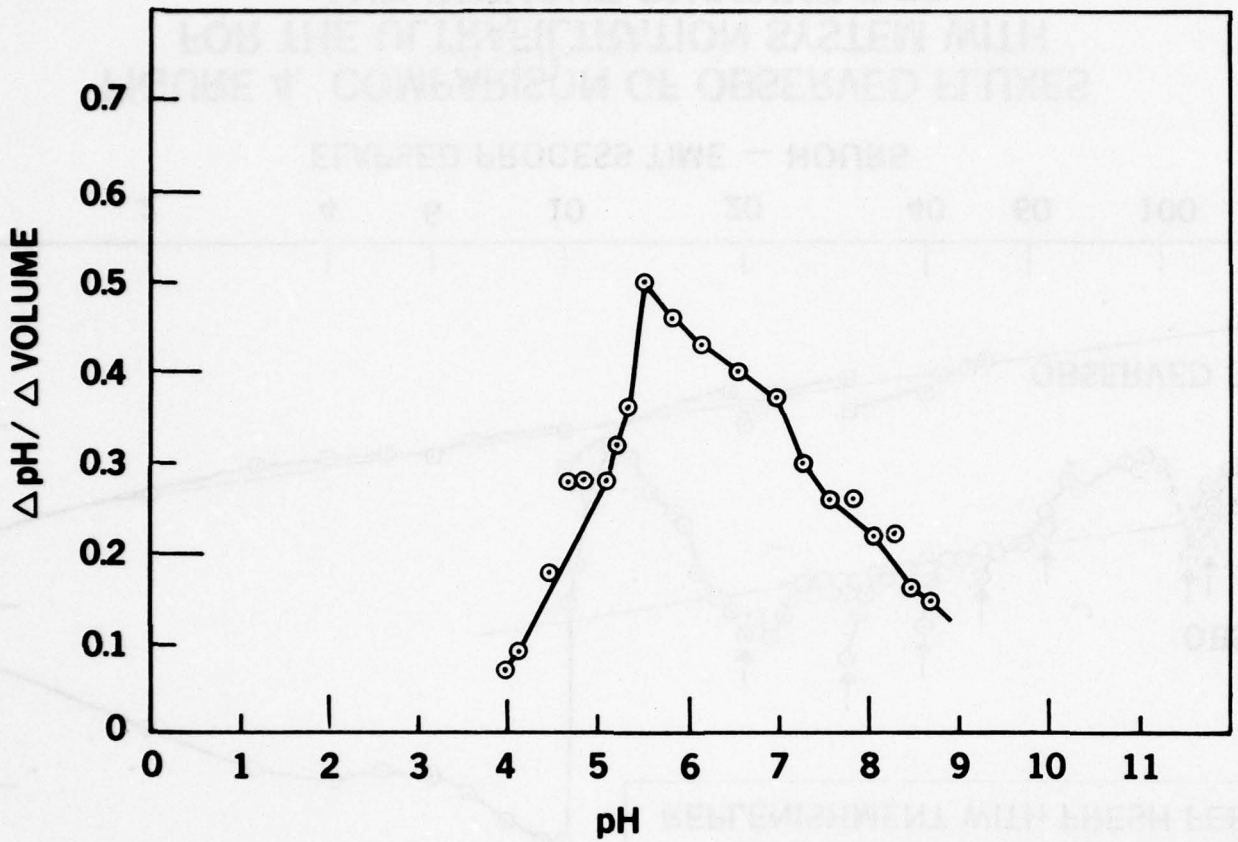

FIGURE 3. DETERMINATION OF THE ISOELECTRIC pH OF A 10%(w/v) RHOZYME P-53 SOLUTION

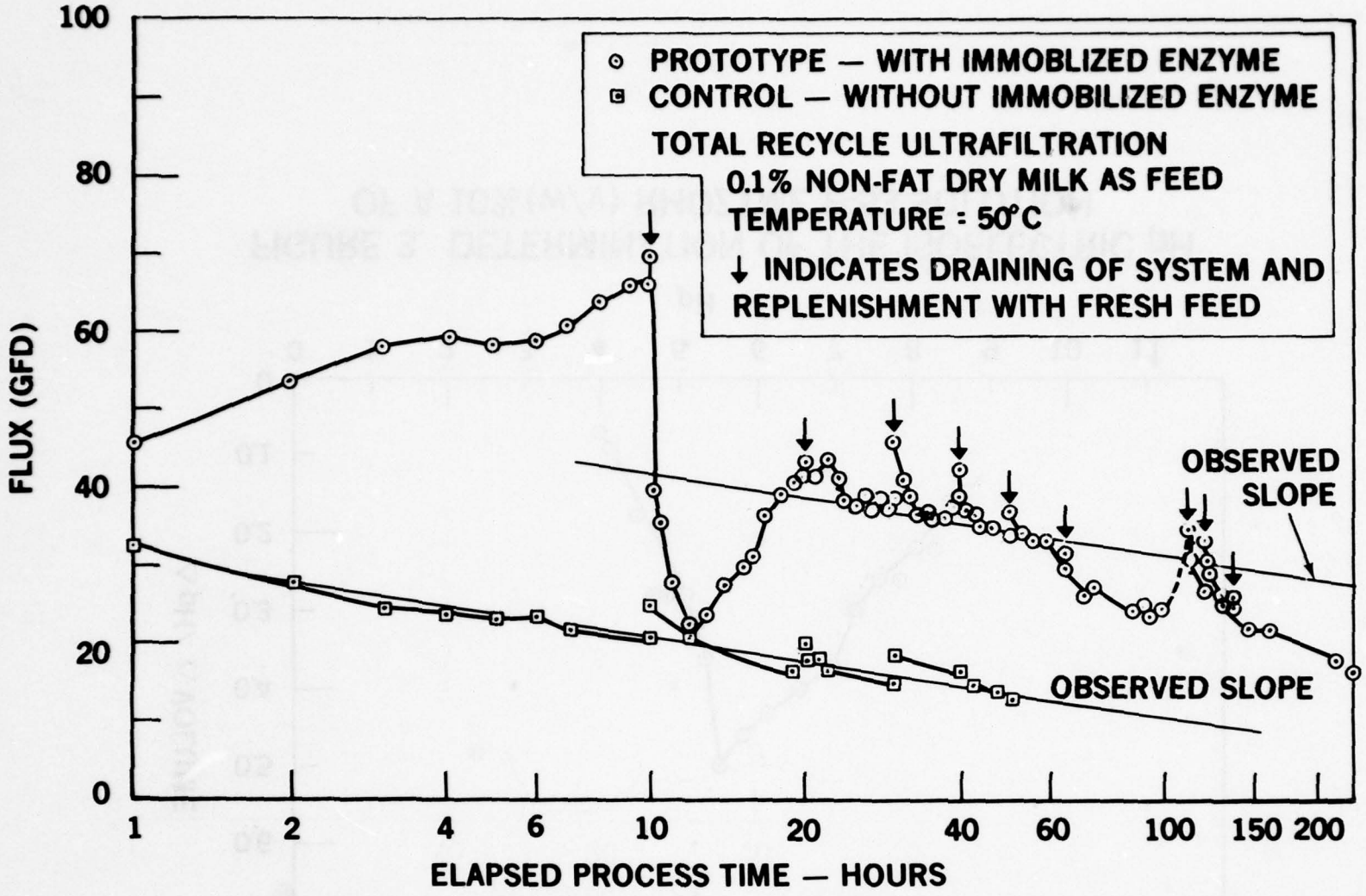

FIGURE 4. COMPARISON OF OBSERVED FLUXES FOR THE ULTRAFILTRATION SYSTEM WITH AND WITHOUT RHOZYME P-53

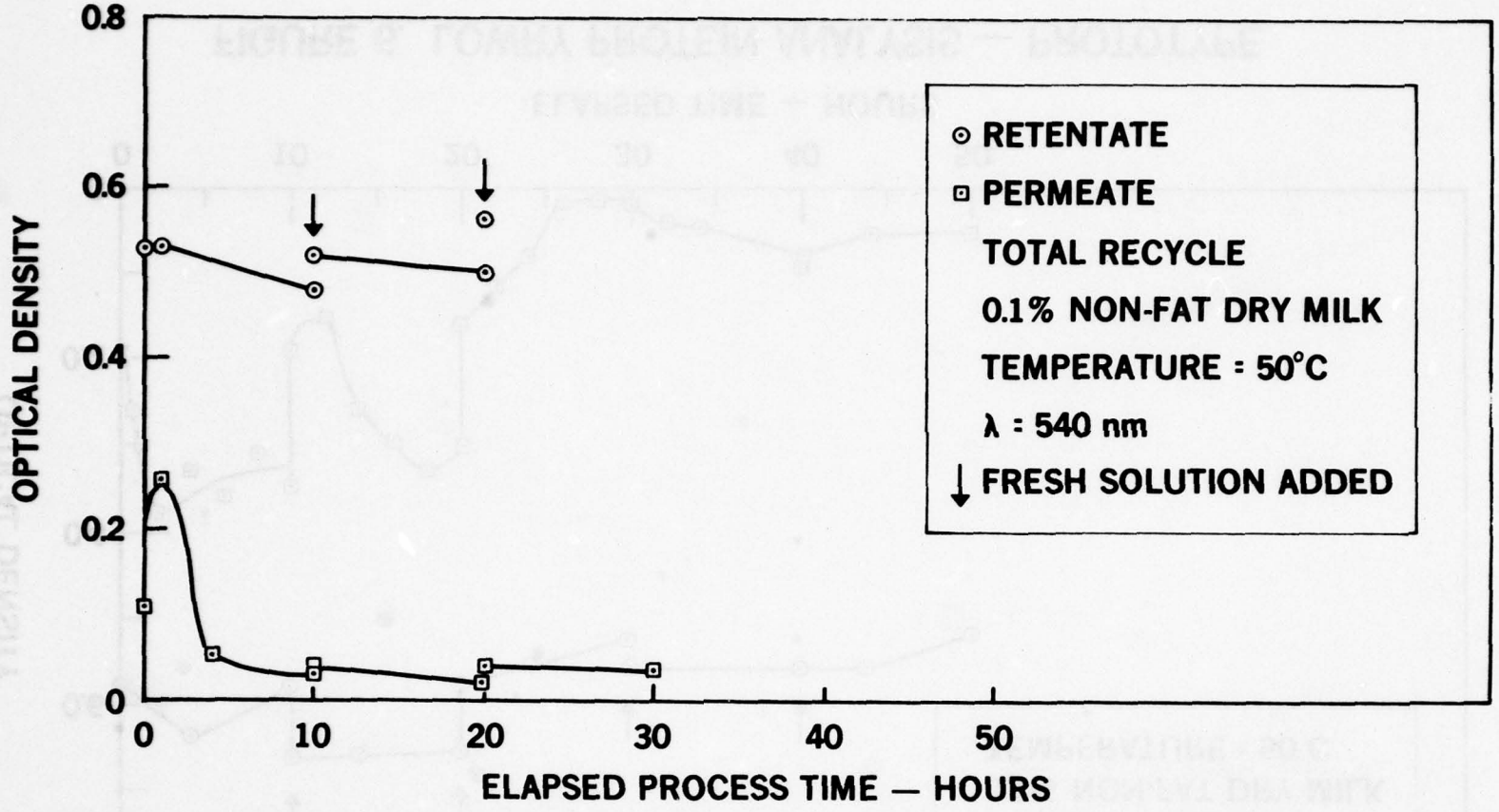

FIGURE 5. LOWRY PROTEIN ANALYSIS — CONTROL

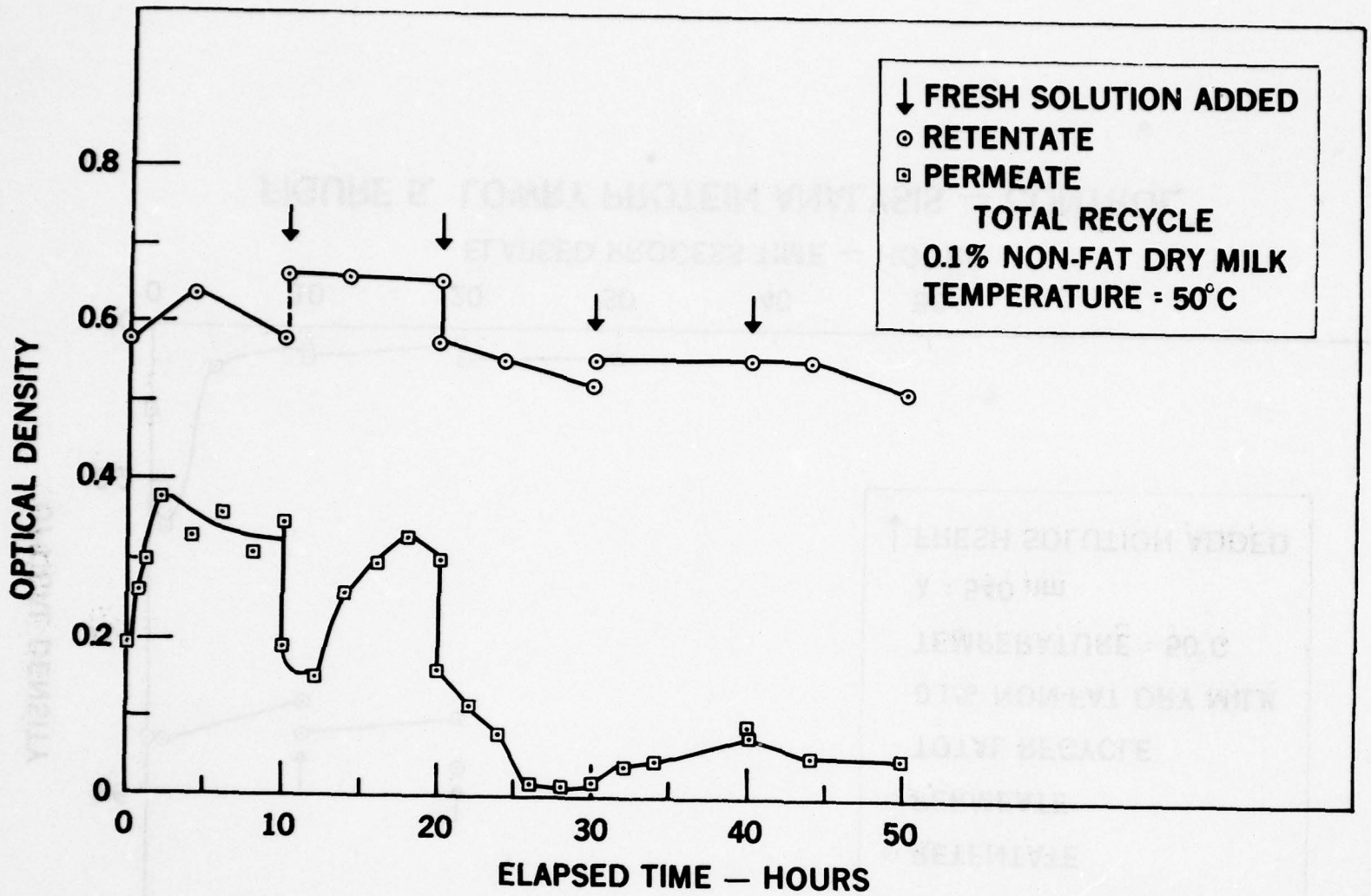

FIGURE 6. LOWRY PROTEIN ANALYSIS — PROTOTYPE

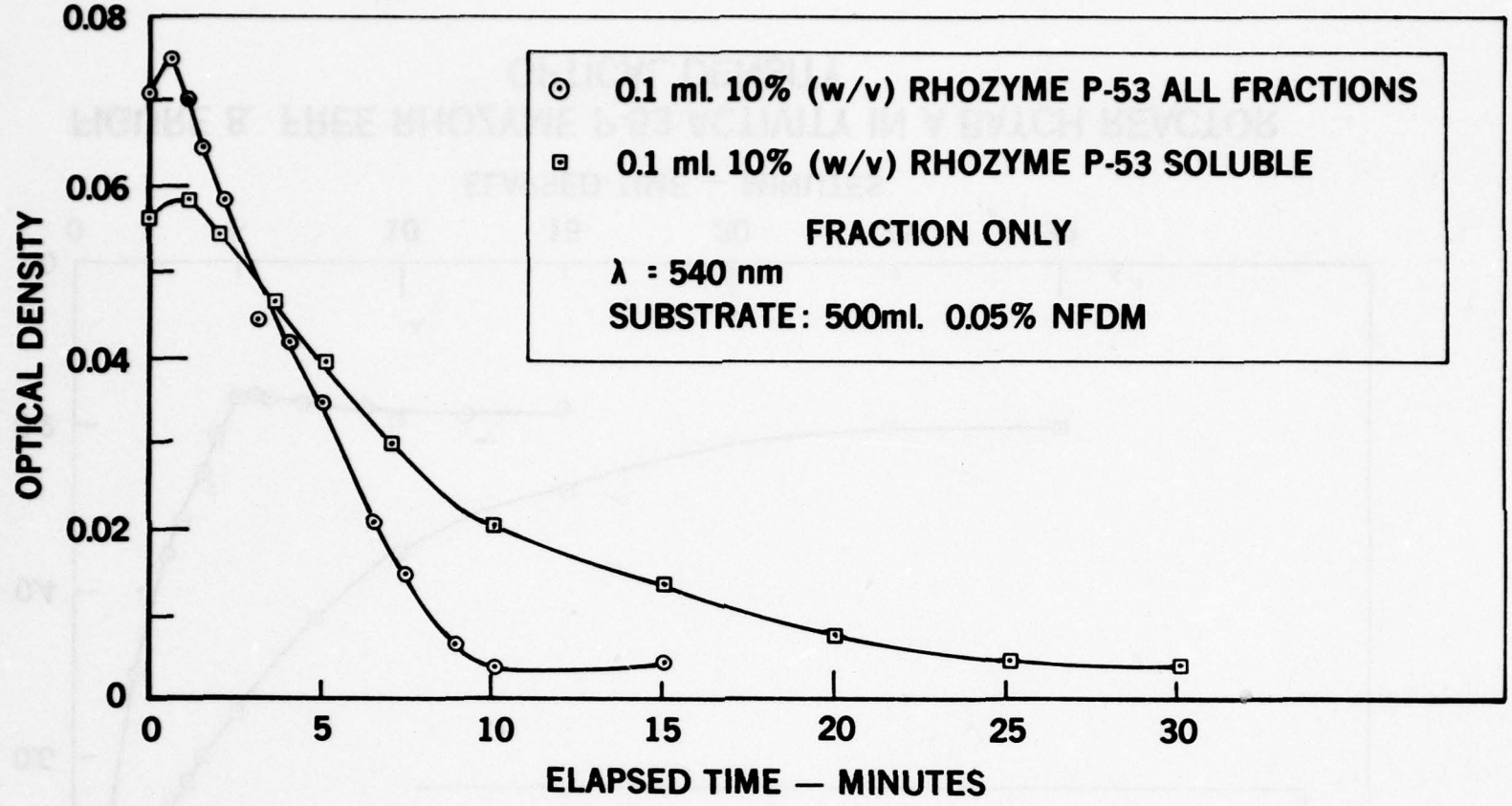

FIGURE 7. FREE RHOZYME P-53 ACTIVITY IN A BATCH REACTOR OPTICAL DENSITY

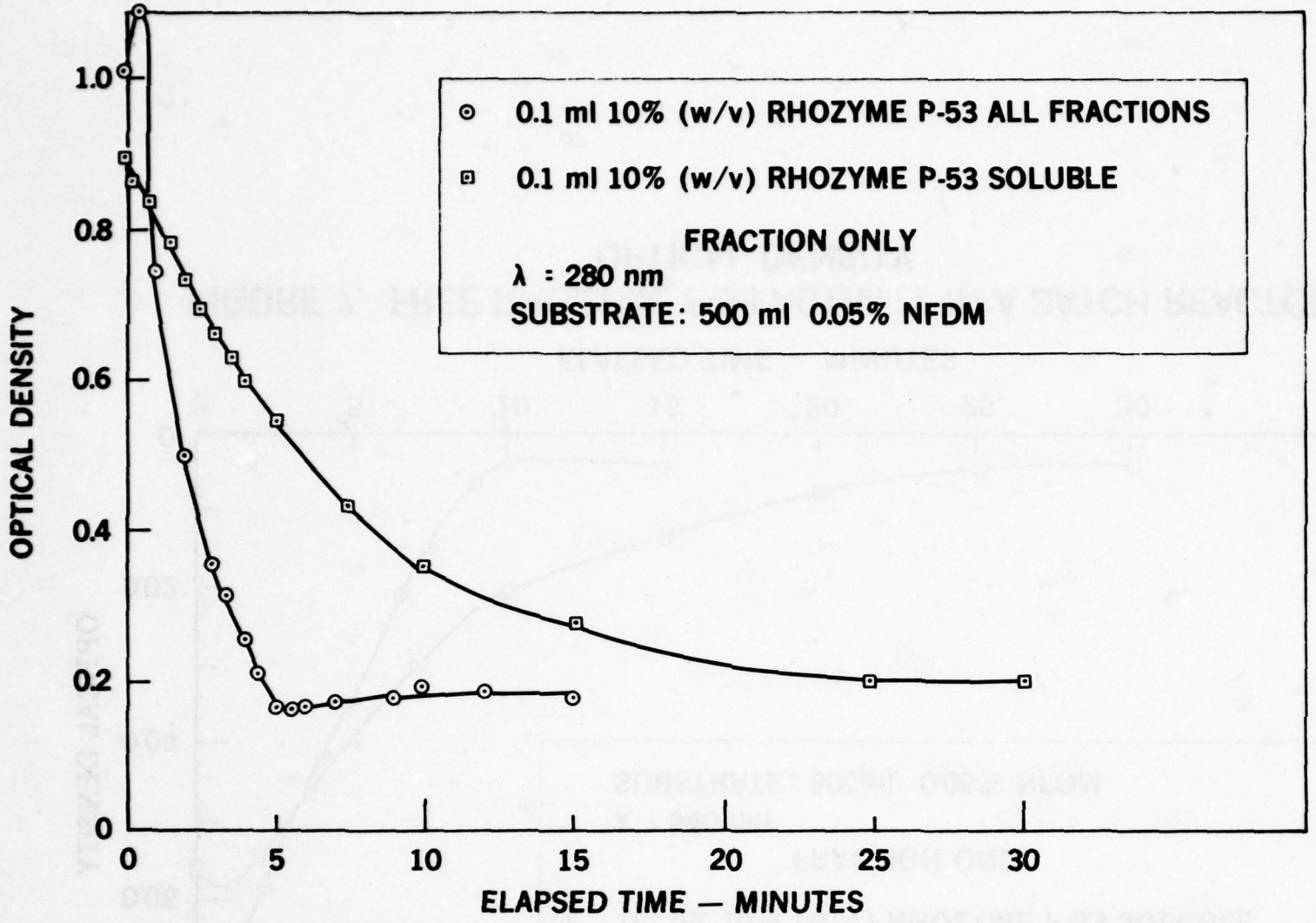

FIGURE 8. FREE RHOZYME P-53 ACTIVITY IN A BATCH REACTOR OPTICAL DENSITY

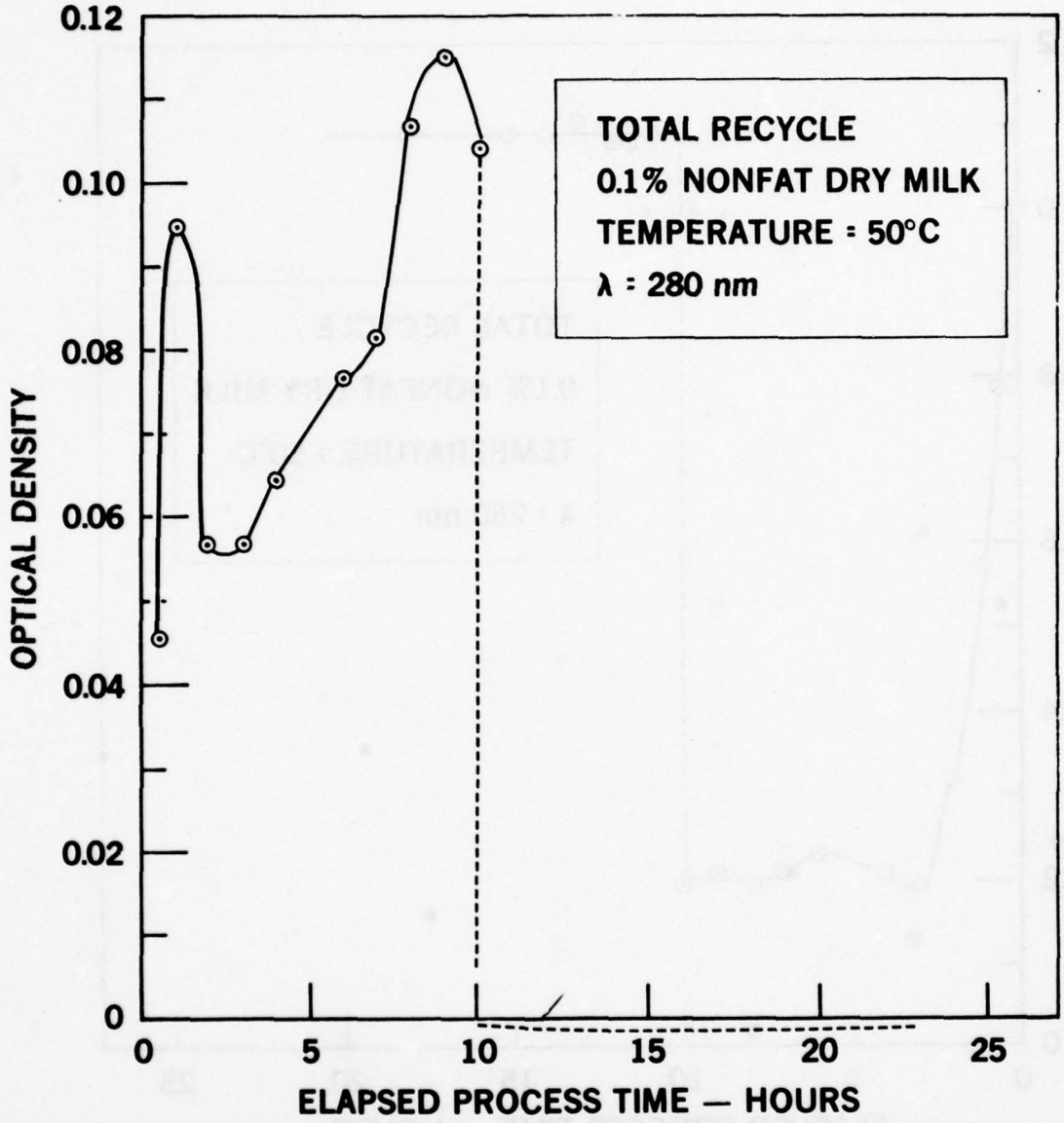

FIGURE 9. MILK PRECIPITATION METHOD OF MEASURING PROTEIN HYDROLYSIS IN A PROTOTYPE SYSTEM

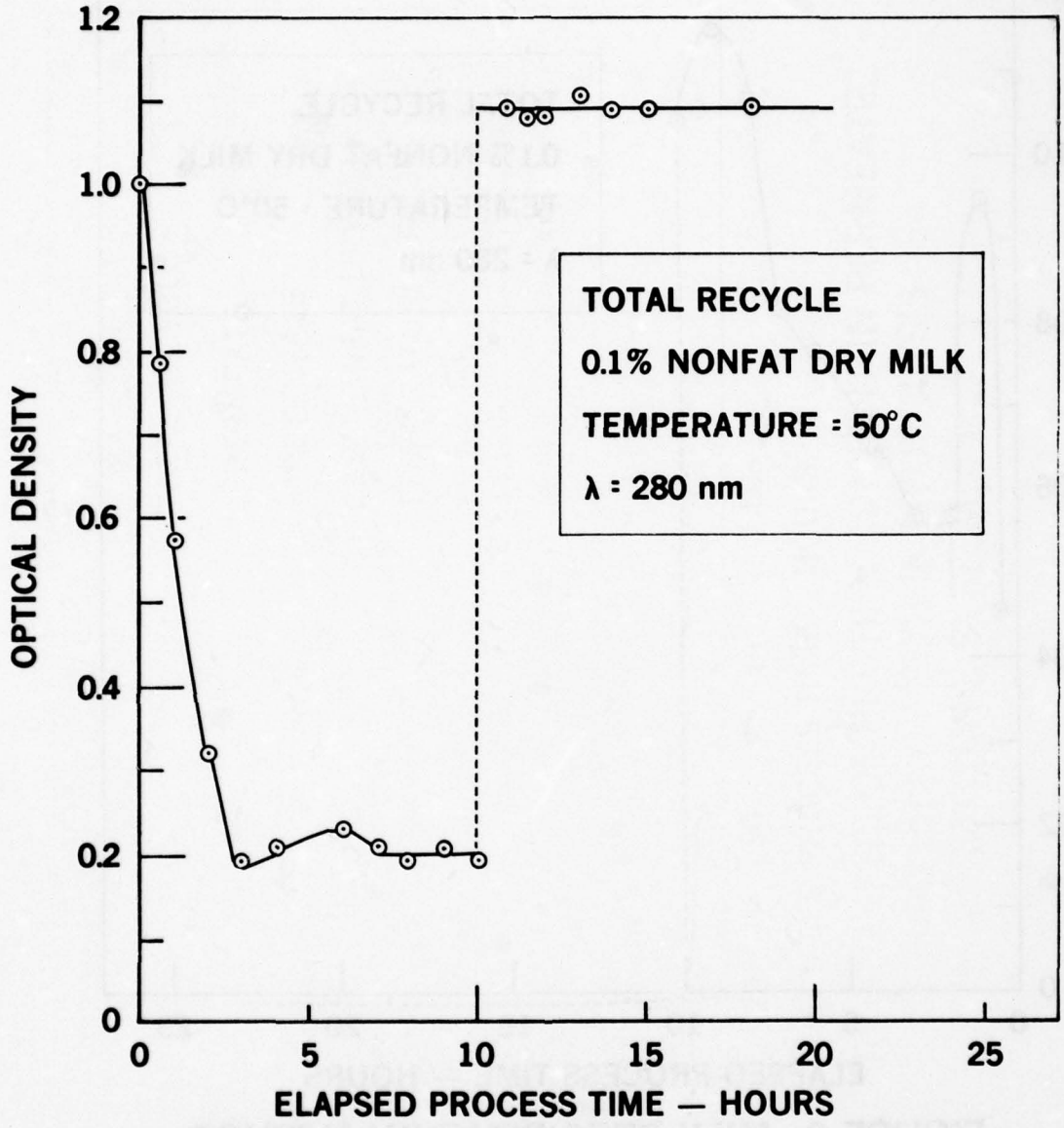

FIGURE 10. OPTICAL DENSITY MEASUREMENT AT λ = 280 nm METHOD OF MEASURING PROTEIN HYDROLYSIS IN A PROTOTYPE SYSTEM

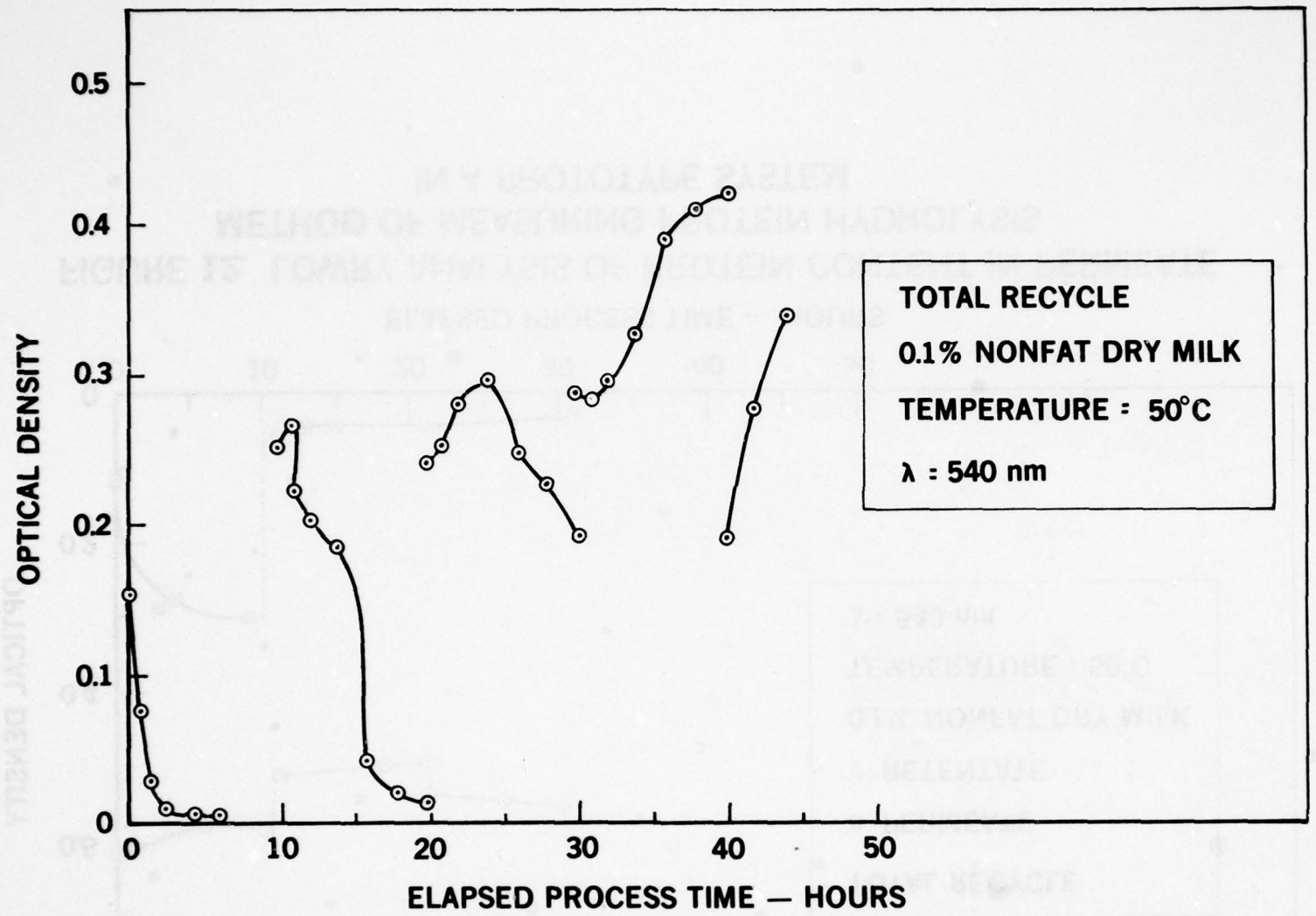

FIGURE 11. OPTICAL DENSITY MEASUREMENT AT λ = 540 nm METHOD OF MEASURING PROTEIN HYDROLYSIS IN A PROTOTYPE SYSTEM

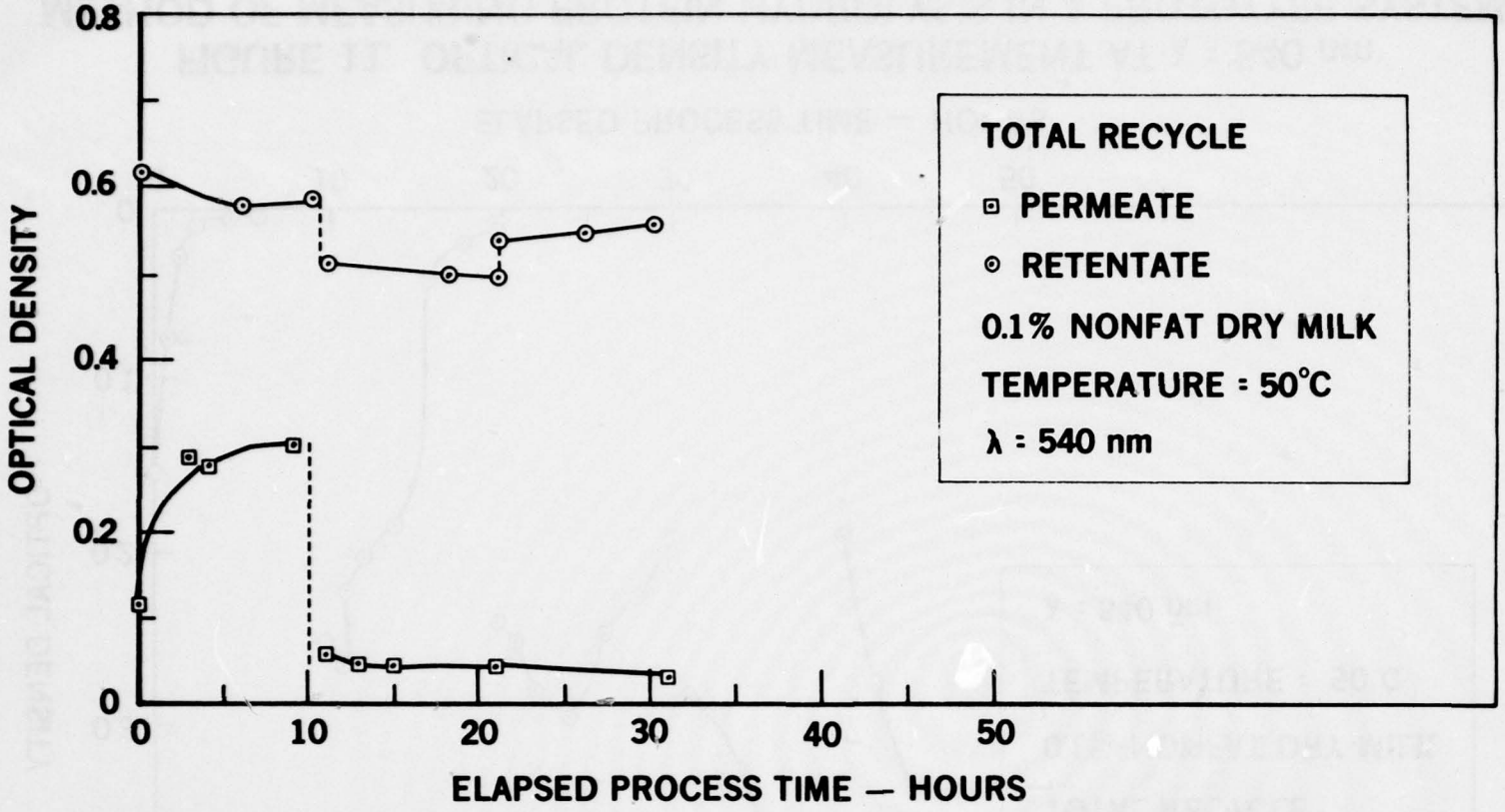

FIGURE 12. LOWRY ANALYSIS OF PROTEIN CONTENT IN PERMEATE METHOD OF MEASURING PROTEIN HYDROLYSIS IN A PROTOTYPE SYSTEM

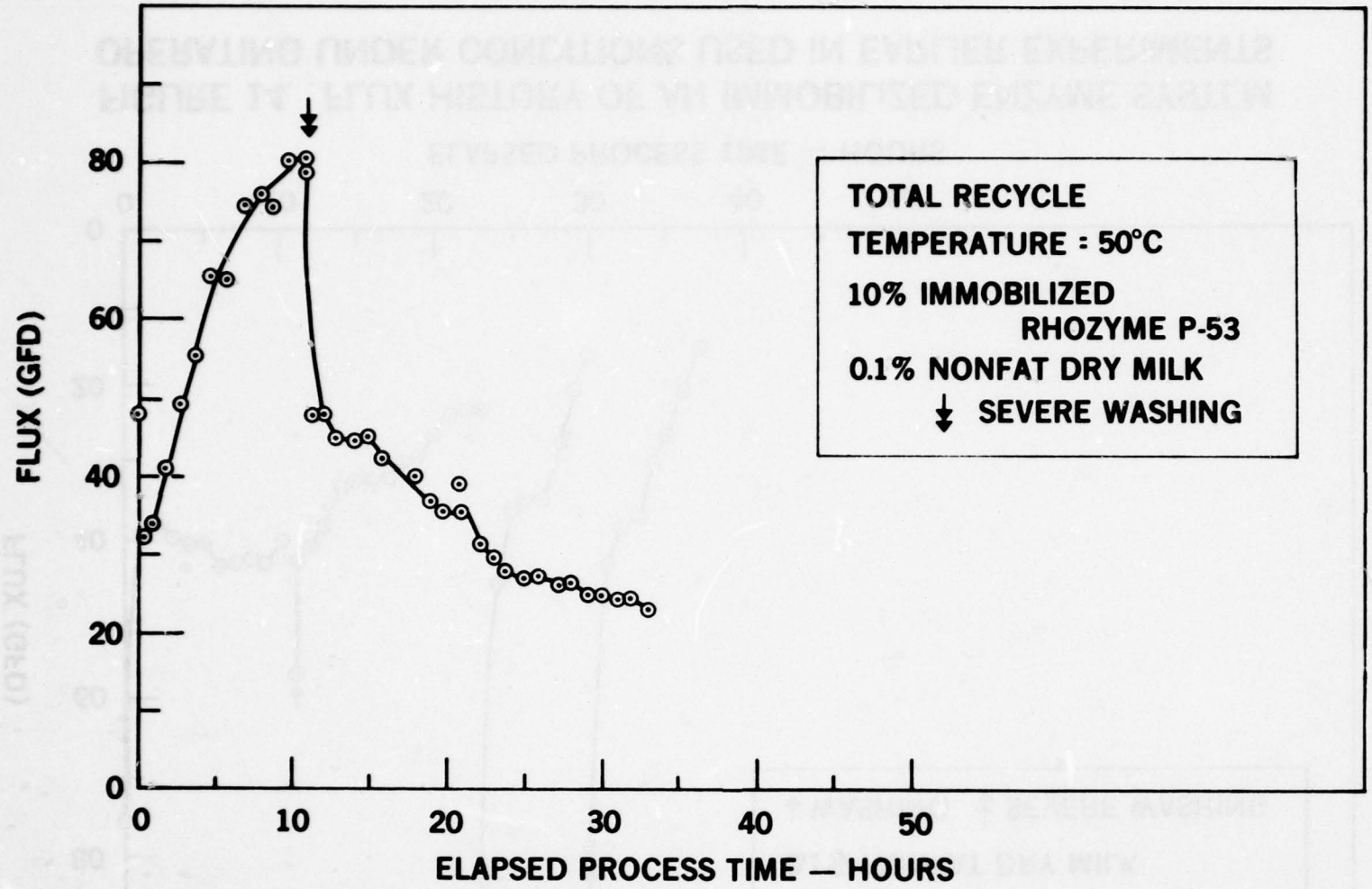

FIGURE 13. PERMEATE FLUX HISTORY OF THE INITIAL PROTOTYPE SYSTEM TESTED

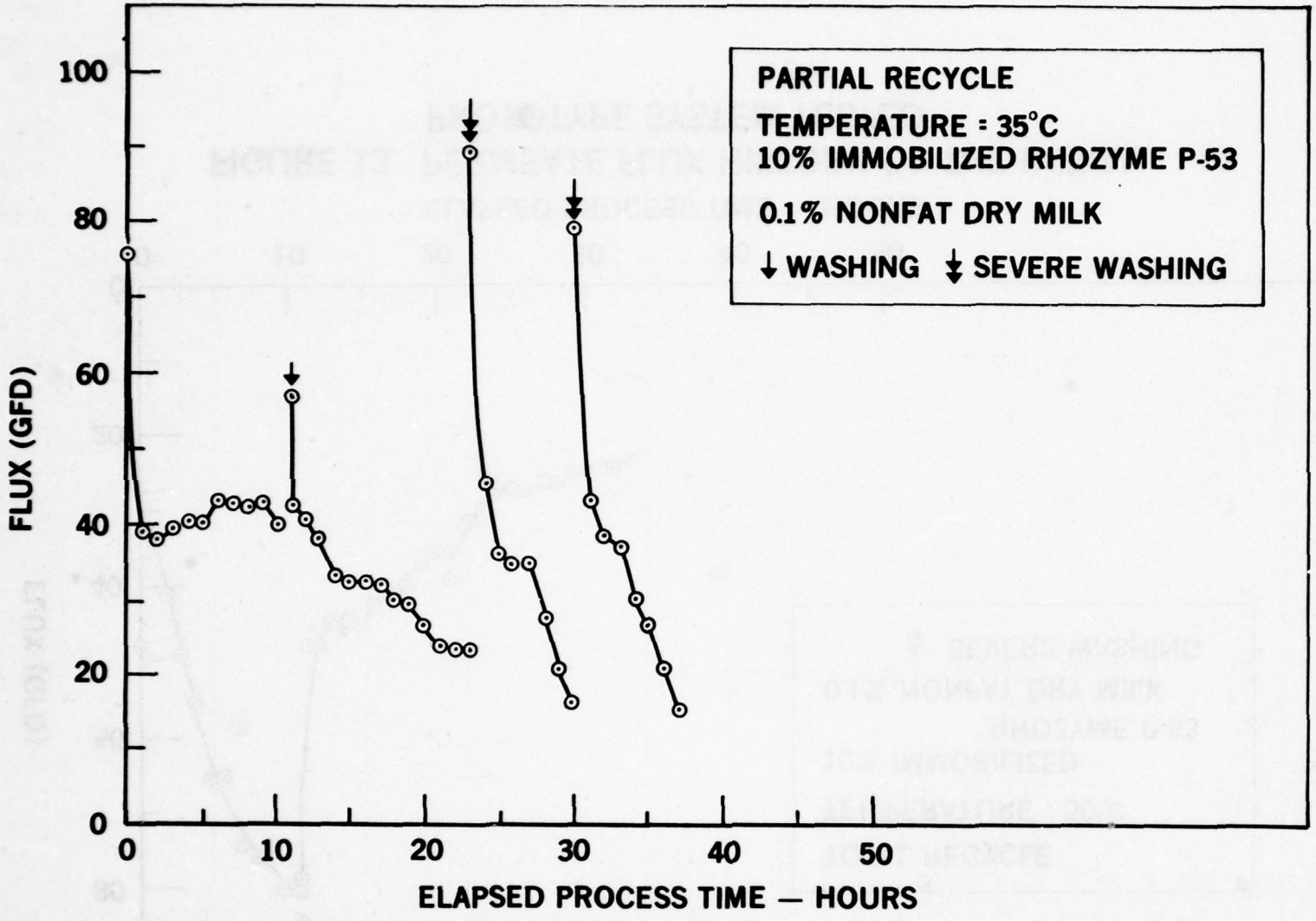

FIGURE 14. FLUX HISTORY OF AN IMMOBILIZED ENZYME SYSTEM OPERATING UNDER CONDITIONS USED IN EARLIER EXPERIMENTS

APPENDIX A
TABULATED RESULTS

Table A-1 Determination of the Isoelectric

pH of Rhozyme P-53 Solution

(Acid titration of 100 ml of a basic 10% (w/w)
Rhozyme P-53 solution.)

pH	0.05M HCl Vol. added (ml)	$\frac{\Delta pH}{\Delta V}$
11.00	2.10	0.038
10.89	4.70	0.060
10.74	7.18	0.063
10.47	10.68	0.095
10.15	13.65	0.117
9.85	16.15	0.124
9.68	17.60	0.113
9.61	18.25	0.100
9.53	19.00	0.100
9.42	20.00	0.120
9.21	21.75	0.124
9.02	23.25	0.140
8.92	24.00	0.130
8.78	25.00	0.150
8.62	26.00	0.170
8.45	27.00	0.160
8.26	28.00	0.220
8.04	29.00	0.220
7.80	30.00	0.260
7.54	31.00	0.260
7.26	32.00	0.300
6.93	33.00	0.370
6.54	34.00	0.400
6.13	35.00	0.430
5.81	35.75	0.460
5.59	36.25	0.500
5.37	36.75	0.360
5.20	37.25	0.320
5.05	37.75	0.280
4.84	38.75	0.280
4.61	40.00	0.180
4.45	41.00	0.100
4.32	42.00	0.100
4.18	43.50	0.095
4.01	45.50	0.075

Table A-2. Control without Immobilized Enzyme
Total Recycle, Temperature = 50° C
0.1% Nonfat Dry Milk

Total Time (hr)	Temp. (°C)	Flux (GFD)	Lowry Protein Analysis O.D. = 540 Retentate	Lowry Protein Analysis O.D. = 540 Permeate	O.D. λ = 280 Retentate Dilution = 0.5/1
0	50	101.8	0.53	0.113	1.0
1/4	49.5	44.4			
3/4	50	34.8	0.53	0.262	1.09
1	50	33.1			
2	50	27.8			
3	50	25.2			
4	50	24.4		0.055	
5	50	22.6			
6	50	23.5			
7	48	21.8			
10	49	20.9	0.48	0.040	
→					
10	49	24.4	0.52	0.033	1.05
11	51	21.8			
12	51	20.9			
19	48	16.5			
20	51.5	18.3	0.50	0.025	1.35
→					
20	48.5	20.0	0.56	0.040	1.21
21.5	48	17.4			
22	50	18.3		0.035	
26	48.5	15.7			
30	48	15.7			
→					
30	46	19.1			
40	51	17.4			
→					
40	50	16.5			
42.5	50	14.8			
47.5	50	14.0			
49	49	13.1			

→ Indicates when system was drained and fresh solution added.

Table A-3. Second Prototype with Improvements

Prototype (2nd Tested) - with Immobilized Enzyme
Total Recycle, Temperature = 50° C
0.1% Nonfat Dry Milk

Total Time (hr)	Temp. (°C)	Flux (GFD)	Lowry Protein Analysis O.D. λ = 540		O.D. λ = 540 Retentate
			Retentate	Permeate	
0	50	88.7	0.54	0.19	0.154
0.25	48	43.5			
0.5	48	39.2		0.26	0.077
0.75	49	45.2			
1	50	46.1		0.30	0.031
2	49	53.9		0.38	0.009
3	48.5	57.4			
4	51	60.0	0.64	0.33	0.007
5	48	58.3			
6	48	58.3		0.36	0.007
7	49	60.9			
8	48.5	62.6		0.31	
9	50	64.4			
10	50	65.3	0.54	0.35	
→					
10	50	69.6	0.66	0.18	
10.25	50	39.2			0.25
10.5	50	36.5			0.245
11	49	27.8			0.240
12	48.5	21.8		0.15	0.20
13	50	24.4			
14	50	27.8	0.65	0.26	0.185
15	49	29.4			
16	49	31.3		0.30	0.04
17	50	37.4			
18	51	39.2		0.33	0.02
19	51	40.9			
20	50	41.8	0.65	0.30	0.015
→					
20	49	43.5	0.58	0.16	0.245
20.25	49	43.5			0.245
20.5	49	42.6			0.250
21	49.5	41.8			
22	50	43.5		0.12	0.280
23	50	41.8			
24	49	37.4	0.56	0.08	0.295
25	51	37.4			
26	50	38.3		0.02	0.249
27	50	37.4			

→ Indicates when system was drained and fresh solution added.

Table A-3 - Continued

Total Time (hr)	Temp. (°C)	Flux (GFD)	Lowry Protein Analysis O.D. λ = 540 Retentate	Permeate	O.D. λ = 540 Retentate
28	50	38.3		0.02	0.229
29	49.5	37.4			
30	49.5	38.3	0.52	0.02	0.190
30	49	46.1	0.56		0.288
30.5	51.5	43.5			
31	51	40.9			0.285
32	50	39.2		0.04	0.297
33	50	36.5			
34	50	36.5		0.05	0.33
35	50	36.5			
36	49	36.5			0.39
37	50	36.5			
38	51	37.4			0.41
39	51.5	38.3			
40	51	38.3	0.56	0.09	0.42
40	50	43.5		0.09	0.19
40.25	48	39.2			
40.5	49	36.5			
41	50	36.5			
42	51	37.4			0.28
43	50	34.8			
44	49	34.8	0.56	0.05	0.34
45	49	34.8			
46	49	34.8			
50	49	33.9			
50	50	37.4	0.52	0.05	0.25
50.5	47	33.1			
51	48.5	32.2			
52	49.5	33.1			
53	50	32.2			
54.5	50	33.1			
64.5	50	29.6			
64.5	50	32.2			
65	50	30.5			
65.75	51	31.3			
68.75	48	26.1			
71.5	52	27.8			
72.75	51	28.7			
86.5	48	24.4			
91.5	53	25.2			
94	48	23.8			
98	49	24.4			
112	48	33.1			
112	44	34.8			

Table A-3 Continued

Total Time (hr)	Temp. (°C)	Flux (GFD)	Lowry Protein Analysis O.D. λ = 540 Retentate	Permeate	O. D. λ = 540 Retentate
112.5	48	33.1			
112.75	47	33.1			
113.25	48	31.3			
113.75	48	33.1			
114.5	50	31.3			
116.5	51.5	29.6			
120.5	45	27.0			
120.5	48	33.1			
122	48	29.6			
127	50	26.1			
132	50.5	25.2			
134	50	26.1			
134.5	50	26.1			
134.5	48	25.2			
134.75	49	24.4			
135.5	50	26.1			
137	49	25.2			
143	46	20.9			
158.5	48.5	19.1			
159.5	48	20.9			
161	48	19.1			
161	50	23.4			
182	50	0			
182	48	15.7			
182.5	50	19.1			
183.5	51	20.0			
184	51.5	19.1			
185	51	20.9			
187	49.5	20.0			
189	49	18.3			
193	49	18.3			
193	47	20.0			
215.5	51.5	18.3			
215.5	50.5	17.4			
239.5	50	16.5			

Table A-4. Free Rhozyme P-53 Activity in a Batch Reactor

Substrate: 500 ml 0.05%(w/v) nonfat dry milk

Enzyme Concentration: 0.1 ml 10%(w/v) solution

Time (min.)	λ = 280		λ = 540	
	All Fractions	Soluble Fraction	All Fractions	Soluble Fraction
0.	1.01	0.90	0.070	0.056
0.25		0.87		0.055
0.5	1.09		0.074	
0.75		0.84		
1.0	0.74		0.069	0.058
1.25		0.79		
1.50			0.064	
1.75				
2.0	0.505	0.741	0.058	0.054
2.5	0.525	0.701		
3.0	0.359	0.665	0.044	
3.5	0.319	0.638		0.046
4.0	0.260	0.603	0.042	
4.5	0.210			
5.0	0.171			
5.5	0.170			
6.0	0.170			
6.5			0.021	
7.0	0.180			0.030
7.5		0.439	0.015	
8.0				
9.	0.185		0.006	
10.	0.195	0.358	0.004	0.020
12.	0.190			
15.	0.180	0.282	0.005	0.014
20.				0.008
25		0.201		0.005
30		0.201		0.004

Table A-5. First Prototype with Improvements

Prototype (1st Tested) with Immobilized Enzyme

Total Recycle, Temperature = 50° C

0.1% Nonfat Dry Milk

Total Time (hr)	Temp. (°C)	Flux (GFD)	Lowry Prot. Anal's O.D. λ = 540 Retentate	Lowry Prot. Anal's O.D. λ = 540 Permeate	O.D. λ = 280 Dilut'n=0.5/1	Milk Precipitation O.D. λ =280	pH
0	48	47.6	0.61	0.116	1.0	0.0	7.2
0.25	49	32.2					
0.50	50	32.2			0.78	0.045	7.2
0.75	50	33.1					7.2
1.0	51	33.9			0.57	0.094	7.3
1.75	49.5	37.4					
2	49	40.9			0.32	0.056	7.2
3	49	49.6		0.287	0.19	0.056	7.5
4	51	55.7		0.279	0.21	0.064	7.3
5	48.5	66.1					7.16
6	48	65.3	0.58		0.24	0.076	7.2
7	51	74.8			0.21	0.081	7.2
8	50	76.6			0.19	0.106	7.27
9	48	74.0		0.305	0.20	0.114	7.29
10	52	80.0	0.59		0.20	0.103	7.29
11	49	80.9					7.33
→							
11	50	78.3	0.51	0.059	1.09		6.92
11.25	49	50.5					6.96
11.30	49	47.9			1.08	< 0	7.01
12	51	47.9			1.08	< 0	7.02
13	49	44.4		0.052	1.11	< 0	7.15
14	48	44.4			1.09		7.20
15	51.5	45.2		0.050	1.09		7.35
16	50	42.6					
18	49.5	40.0	0.50		1.09		7.45
19	49	36.5					
20	48	34.8					
21	50.5	34.8	0.50				7.45
→							
21	49	39.2	0.54	0.047	1.1		6.95
22	48	31.3			1.2		6.98
23	49	29.6					7.1
24	48	27.8			1.25		7.22
25	48	27.0					7.22
26	48	27.0	0.55		1.3		7.25
27	48	26.1					7.25
28	48	26.1			1.35		7.30
29	49	25.2					7.32
30	50	25.2	0.56		1.35		7.32
31	49.5	24.4		0.038			
32	49	24.4					
33	48	23.5					

→ Severe washing with water.

→ System drained and fresh solution added.

Table A-6. Prototype from Earlier Experiments
Partial Recycle, Temperature = 35° C
0.1% Nonfat Dry Milk

Total Time (hr)	Temp. (°C)	Flux (GFD)	Tank Volume (Gal.)
0		76.6	20
1		39.2	17
2		38.3	15.5
3		40.0	14
4		40.9	13
5		40.0	11.5
6		43.5	10
7		43.5	9
8		42.6	8
9		43.5	7
10		40.0	6
11		42.4	5
→			
11		56.6	20
12		40.9	19
13		39.2	18
14		33.9	18
15		33.1	17.5
16		33.1	16.5
17		33.1	15.5
18		30.5	15
19		30.5	14.5
20		27.0	13
21		21.8	13
22		21.8	13
23		21.8	13
→			
23	35	89.6	20
24	33	46.1	16.5
25	32	36.5	13.5
26	34	35.7	12
27	37	35.7	10.5
28	35	27.0	8.5
29	35	20.9	7.5
30	37	13.1	7
→			
30	36	80.0	20
31	34	43.5	16.5
32	34	39.2	15
33	36	38.3	12.5
34	34	30.5	10
35	35	27.0	9
36	34	20.9	8
37	35	15.7	7.5

→ Washing with water Extended washing with water

Table A-7. History of Membrane E

Date	Activity
9/12/77	Immobilize 2.5% Rhozyme P-53
9/14 - 9/17	Prototype run under old experimental conditions for 37 hours
9/22	Clean membrane with Chlorox solution for 2 hours.
9/23	Immobilize 10% Rhozyme P-53.
9/24 - 9/26	First prototype with new operating conditions tested for 33 hours.
9/26 - 9/27	Clean membrane with Chlorox solution for 3 hours.
9/27 - 9/30	Run control under new operating condition for 49 hours.
10/3 - 10/4	Clean membrane with Chlorox solution for 11 hours.
10/5	Immobilize 10% Rhozyme P-53.
10/6	Discover that immobilization of 10/5 was insufficient and so immobilization procedure was repeated for 10% Rhozyme P-53.
10/7 - 10/17	Run second prototype under new conditions for 240 hours.

APPENDIX B - INTERIM REPORT (MARCH 1977)

Rutgers - The State University of New Jersey
College of Engineering
Bureau of Engineering Research
PO Box 909
Piscataway, NJ 08854

(Progress Report N-0022)

AN ENZYME APPROACH TO THE PREVENTION AND REMOVAL OF GELATINOUS FILMS IN RAW-SEWAGE ULTRAFILTRATION SYSTEMS

by

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Principal Investigators

and

Professor Feng-Chi Hsieh, Assistant Research Professor

and

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Submitted to:

Mr. Lynne R. Harris
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BACKGPOUND

As reported in December 1976 (Appendix C), many facts were established; the most important of these was that a protease, Rhozyme B-6, could be immobilized easily by a vacuum sorption technique on Abcor HFM membranes. Furthermore, this enzyme was both able to remove a raw sewage gelatinous layer from these membranes in its free solution form and to improve flux rates of protein solutions (casein, nonfat dry milk (NFDM), and raw sewage) in its immobilized form when used in the Amicon Diaflo Apparatus. In some cases, improvements in flux were as high as 350% when the immobilizedenzyme membranes (IEM) rather than untreated membranes were used. From these observations, it was concluded that Rhozyme B-6 was directly responsible for the prevention of gelatinous layer build-up during ultrafiltration of protein solutions, which in turn enabled significant increases in permeation to occur.

EXPERIMENTAL

APPARATUS. A sketch of the continuous-recycle apparatus is shown in Figure 1. Referring to Figure 1, protein solutions are added to a stainless steel holding tank (11) and circulated at a pressure of 40 lb/in^2g. During the experiment, the solution passes across the membrane surface (6) and is either collected as permeate through a tube connected at the lower section of the membrane cartridge (7) or is returned to the tank. A bypass section is also included so that solutions can be returned to the tank without passing through the membrane.

IMMOBILIZATION PROCEDURE. Rhozyme B-6 was used in the continuous-recycle experiments because it had the best activity in the experiments with the Amicon Diaflo Apparatus (see Appendix C). Approximately 1½ liters of 10%

(W/V) Rhozyme B-6 solution was prepared and filtered. A vacuum was created in the Abcor HFM tube by subjecting it to a vacuum of 25.6 inches of Mercury for 10 minutes. The enzyme solution was then pulled into the membrane and allowed to sit for 24 hours. The excess enzyme solution was drained and 75 gallons of tap water flushed through the module. The history of the protein concentration in the permeate is shown in Figure 2. After loosely bound enzymes leached out with water, the system was ready for use.

BASIC EXPERIMENTAL PROCEDURE. The tank was initially filled with 20 gallons of a 0.1% (W/V) protein solution. The system was started by application of a pressure of 40 lb/in^2g, an initial flux was noted, and a permeate sample was collected for protein analysis (Lowry Method). After ½ hour, a sample was taken from the holding tank for a protein analysis. (When raw sewage is used, a sample is also taken from the holding tank (retentate) at the same time so that the solids content may also be determined.) Permeation rates were noted every ½ hour, and samples for the protein analysis were collected every hour. It is essential that the temperature remain constant. This was accomplished by adding enough ice approximately every ½ hour to maintain the desired temperature. When the volume of the tank reached 7.5 gallons, the final membrane flux, permeate samples, and retentate samples were taken. The system was then shut off and ready for cleaning.

BASIC MEMBRANE CLEANING PROCEDURE. At the termination of the experiment, the contents in the tank and piping were emptied. The system was filled with water, flushed, and redrained. This procedure was repeated until the tank was free of all particulate matter. Then, 500 milliliters of commercial Chlorox were added to 25 gallons of water and the system was

flushed (recycling) for 45 minutes. This method of cleaning was thorough enough to allow the regenerated water flux to be at least as high as the water flux before any experiment or immobilization.

RESULTS

RESULTS FOR THE CONTINUOUS RECYCLE ULTRAFILTRATION OF NONFAT DRY MILK. Figure 3 is the flux curve when 0.1% nonfat dry milk (NFDM) solution is ultrafiltered through a membrane with no enzyme. The initial flux is 56 gal/ft^2/day but drops within 20 minutes to a steady state value of 16 gal/ft^2/day. In the case of the 10% (W/V) Rhozyme B-6 IEM (Figure 4), the initial permeate flux is 76 gal/ft^2/day, drops slightly below the steady state flux of 50 gal/ft^2/day--for approximately 1 hour, then rises to this steady state value.

The steady state IEM membrane flux of the continuous recycle system is 233% greater than the flux obtained from the control. It is clear that the immobilized Rhozyme B-6 is actively degrading or preventing the formation of the gelatinous layer on the membrane. The fact that the protein concentration in the tank increased 123% over a period of 3 hours indicated the success of the IEM system in concentrating the NFDM solution.

Figure 5 further demonstrates that immobilized Rhozyme B-6 prevents the formation of a gelatinous layer and maintains higher permeation rates than a membrane without immobilized enzyme.

RESULTS FOR THE CONTINUOUS RECYCLE UTLRAFILTRATION OF RAW SEWAGE. When raw sewage is ultrafiltered by a membrane without immobilized enzyme (Figure 1) the initial flux is 52 gal/ft^2/day. This flux drops to 33 gal/ft^2/day within ½ hour and reaches a steady state flux of 30 gal/ft^2/day within 1 hour of the start of the experiment. The initial flux of the IEM with raw sewage is 55 gal/ft^2/day as shown in Figure 7. This experiment was

run for 22 hours. After 7 hours of evaluation, it was decided to maintain the tank level at 12.5 gallons. A steady state flux of 42 gal/ft^2/day existed for the last 10 hours of the experiment.

In comparison with the control (ultrafiltration of raw sewage), the Rhozyme B-6 IEM ultrafiltration of raw sewage showed a 44.8% improvement at steady state flux. Again, it is evident that the immobilized protease, Rhozyme B-6, is an active factor in the removal of the gelatinous layer from the membrane surface.

COMPARISON OF RESULTS FOR NONFAT DRY MILK AND RAW SEWAGE ULTRAFILTRATIONS. The improvement of flux during the ultrafiltration of raw sewage is not as dramatic as in the case of the nonfat dry milk; there are several factors which must be considered before definite conclusions can be made. These include the lack of homogeneity and solids content variations in raw sewage samples.

Raw sewage is much less homogeneous than the NFDM solutions, and yet, it has never been subjected to physical or chemical treatment before entering the continuous recycle system. The December progress report explained that drastic increases in flux resulted when the raw sewage was prescreened with multiple layers of cloth. It is believed that if recirculated through the bypass for ½ hour, the raw sewage will become macerated enough to lay down more evenly on the membrane and thus, become more available for direct enzymatic degradation.

Another unique difficulty in ultrafiltering raw sewage lies in the present inability to control the solids content and quality of the sewage. As was shown in the December report, increase in the solid content in sewage

decreases the flux considerably. It is worth noting that in the raw sewage experiments most of the initial solids content has been 20 to 50% higher than in the milk runs.

SUMMARY

APPARATUS. A continuous recycle system has been designed and built and has proved to be suited to the unique needs of these particular experiments. In this apparatus, protein solutions may pass through a membrane cartridge under a pressure of 40 lb/in^2g and returned to the tank or may be recycled back to the tank directly. Permeate samples can be measured in terms of flux and can be collected easily. Immobilization of enzymes on the membrane is also accomplished without difficulty. Membrane cartridges can be cleaned completely or replaced easily. In short, the overall design of the apparatus has met all of the necessary requirements of this experiment.

SUPERIORITY OF THE RHOZYME B-6 IMMOBILIZED SYSTEM. The apparent superiority of the enzyme immobilized membranes to the membranes without enzyme with respect to flux is the most important conclusion to be reported. With 0.1% (W/V) milk solutions, the IEM system is 233% better. Membrane flux of raw sewage solutions is improved 45% when the immobilized enzyme membrane is used rather than one without enzyme. This positive experimental evidence is essential when evaluating the feasibility of this entire project. At this time, the experimental results support the continuation of this investigation for the immobilized enzyme membrane ultrafiltration of raw sewage.

OBJECTIVES FOR NEXT QUARTER (MARCH-MAY)

The objectives for the next quarter will be to repeat the previous milk

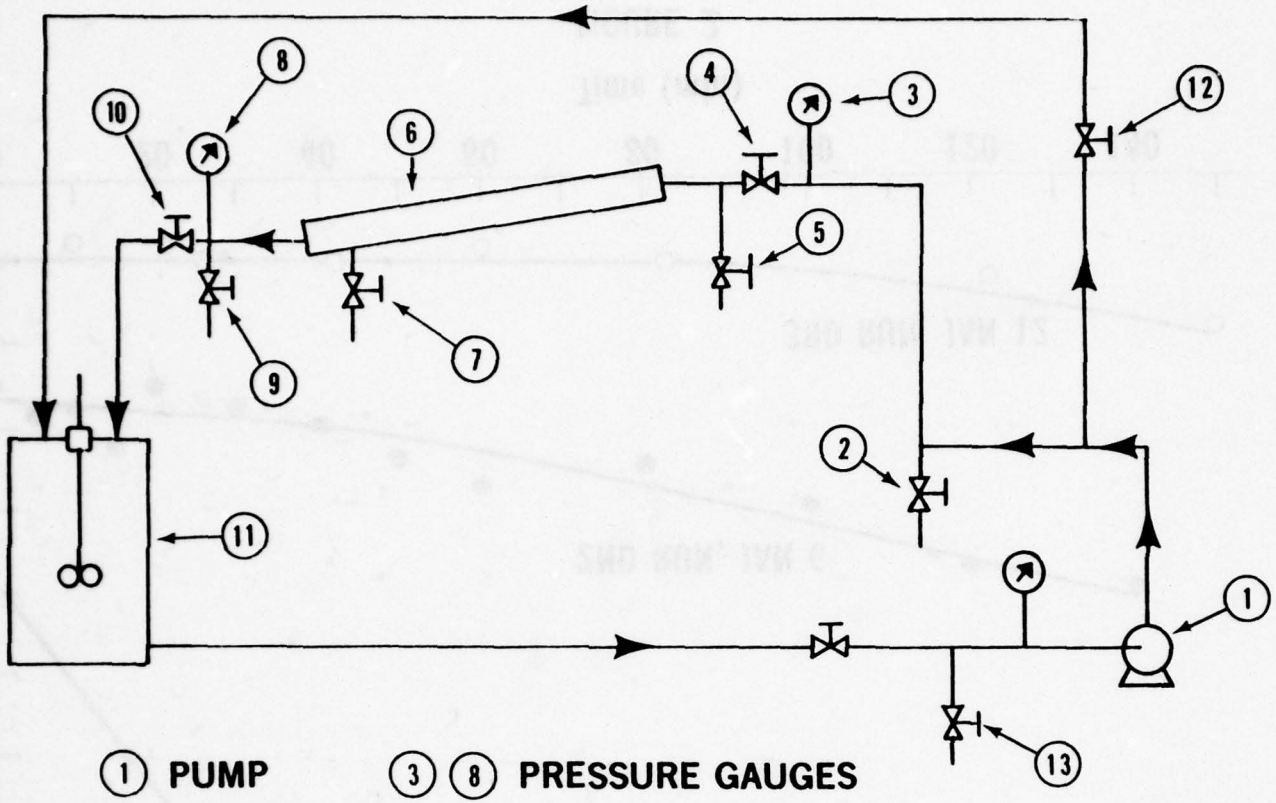
and raw sewage runs in order to establish the degree of reproducibility of the results.

With the added information expected in this next quarter we will be ready to submit patent disclosure forms to the Research Corp.

We will install the new tube in order to commence our next set of experiments from a favorable starting point (i.e., fresh membrane). The membrane will be thoroughly checked for water flux as the basic control over a substantially longer period of time.

STAFFING

Dr. Burton Davidson and Dr. Shaw Wang are the project directors of this investigation. Dr. Fenchi Hsieh is an assistant research professor assigned to this project. Carrie Gillespie has now been officially appointed as a research assistant and is presently working toward her master's degree in the Rutgers University Department of Chemical and Biochemical Engineering. Eileen Hao and Michael Huang are assisting the project on an hourly basis. Both are graduate students in the Rutgers Food Science Department. An undergraduate chemical engineering student, Chris Lashman, is assisting with some of the operational runs on weekends.



(1) PUMP (3) (8) PRESSURE GAUGES

(2) (4) (5) (9) (10) (12) (13) VALVES

(6) MEMBRANE (7) PERMEATE LINE

(11) TANK

FIGURE 1. PROTOTYPE ULTRAFILTRATION UNIT

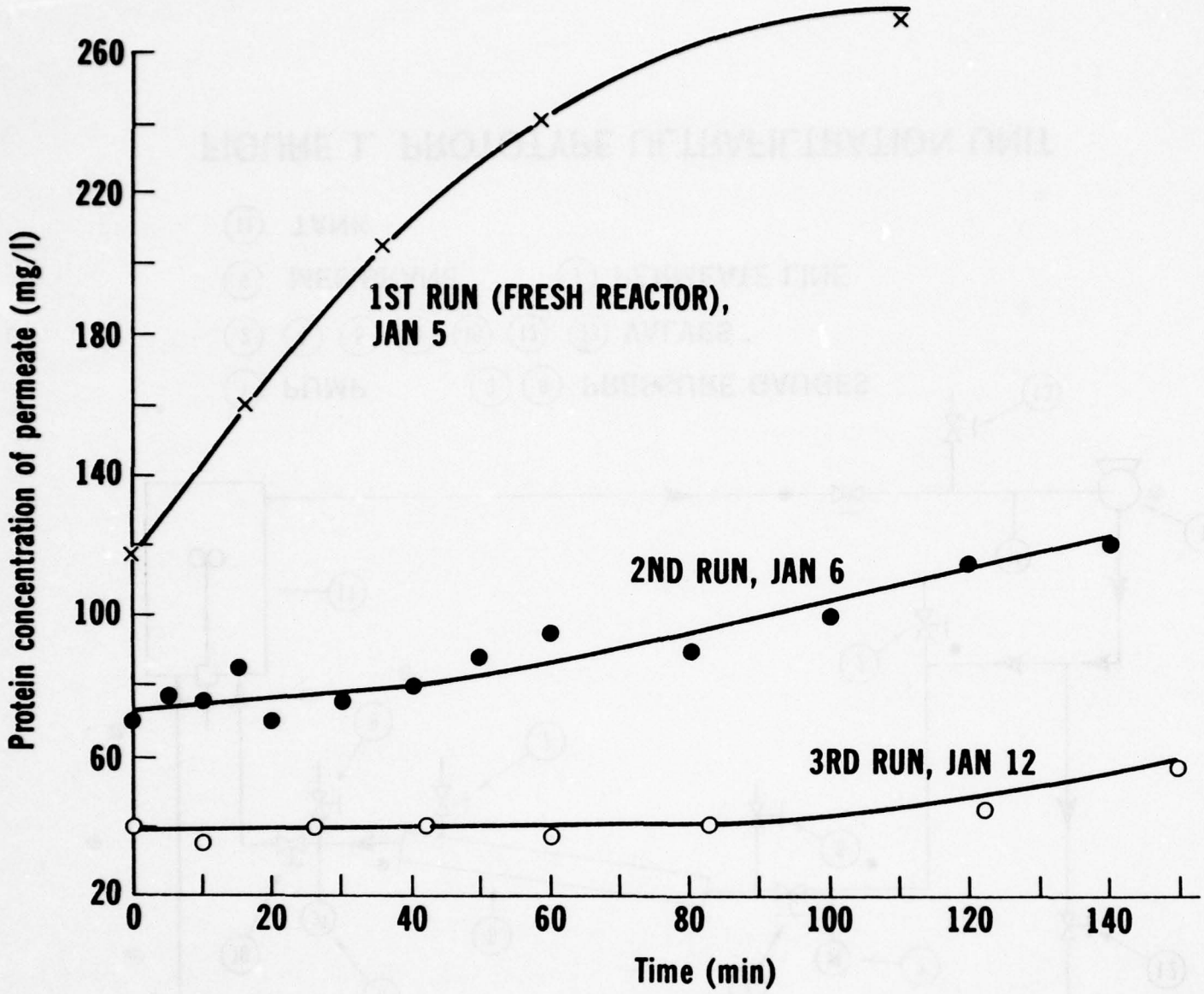


FIGURE 2

PROTEIN CONCENTRATION OF PERMEATE DUE TO ENZYME LEACHING OUT

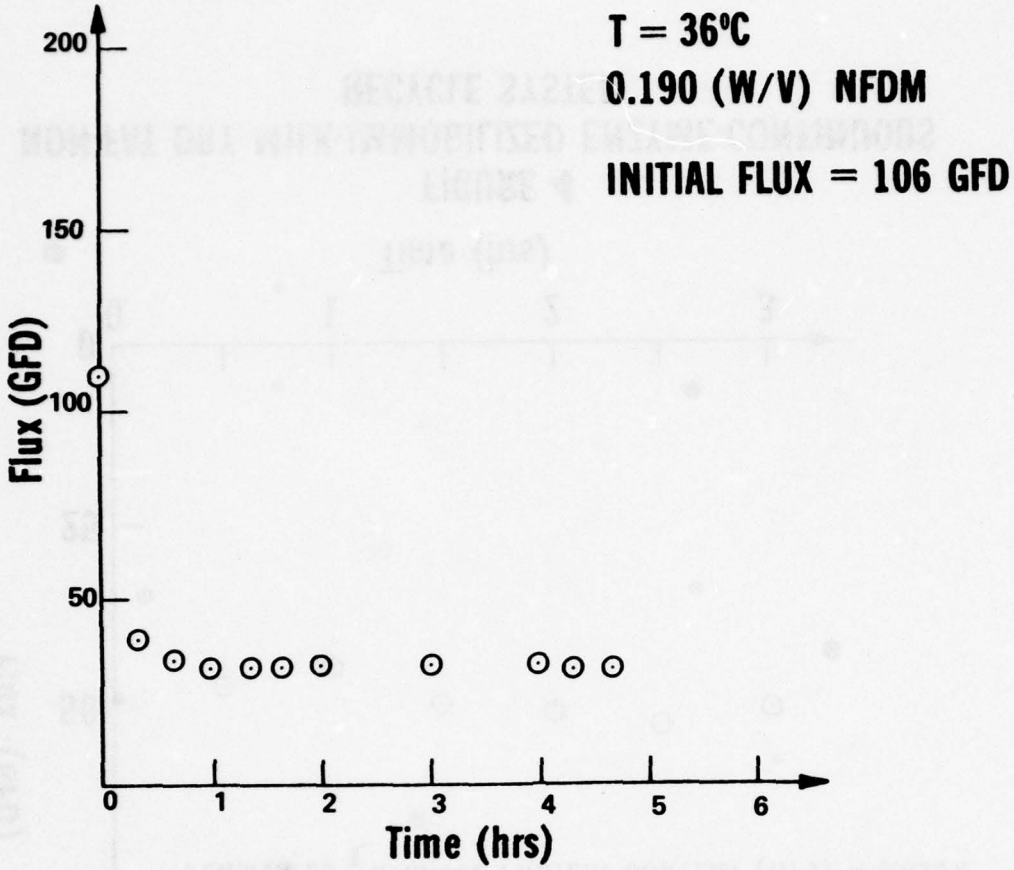


FIGURE 3
NON-FAT DRY MILK-CONTROL–CONTINUOUS RECYCLE SYSTEM

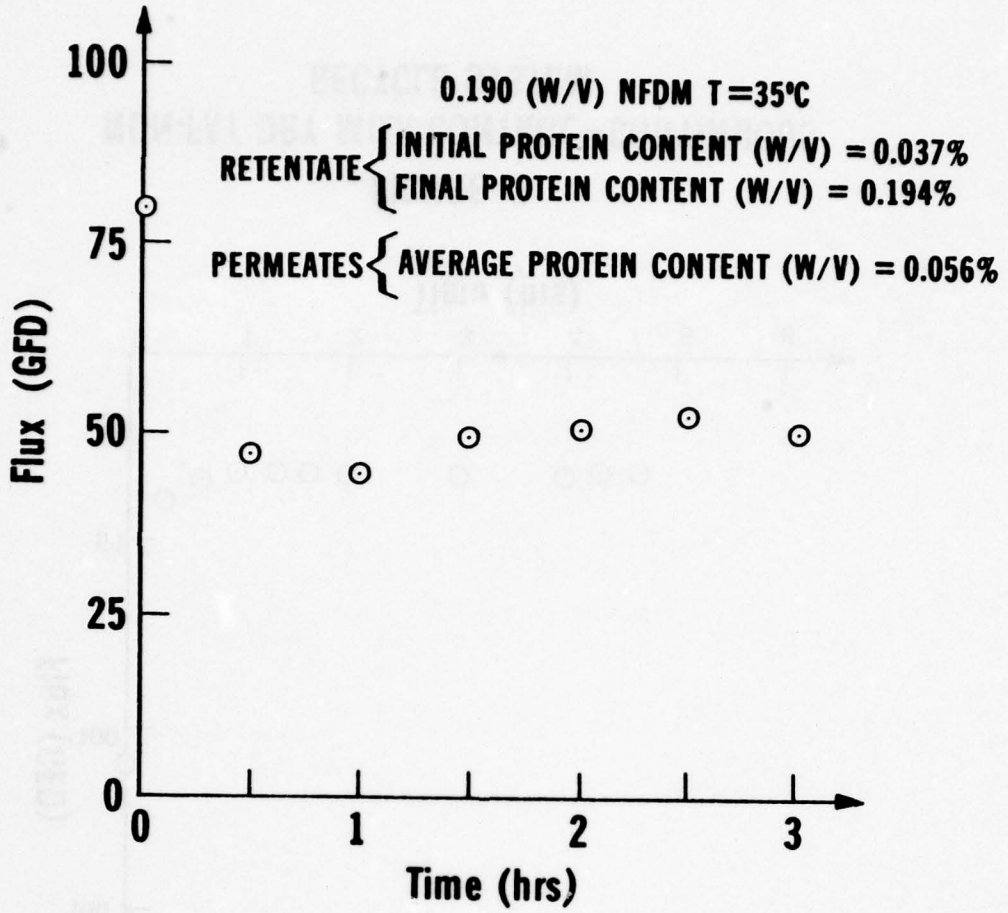


FIGURE 4
NON-FAT DRY MILK-IMMOBILIZED ENZYME-CONTINUOUS RECYCLE SYSTEM

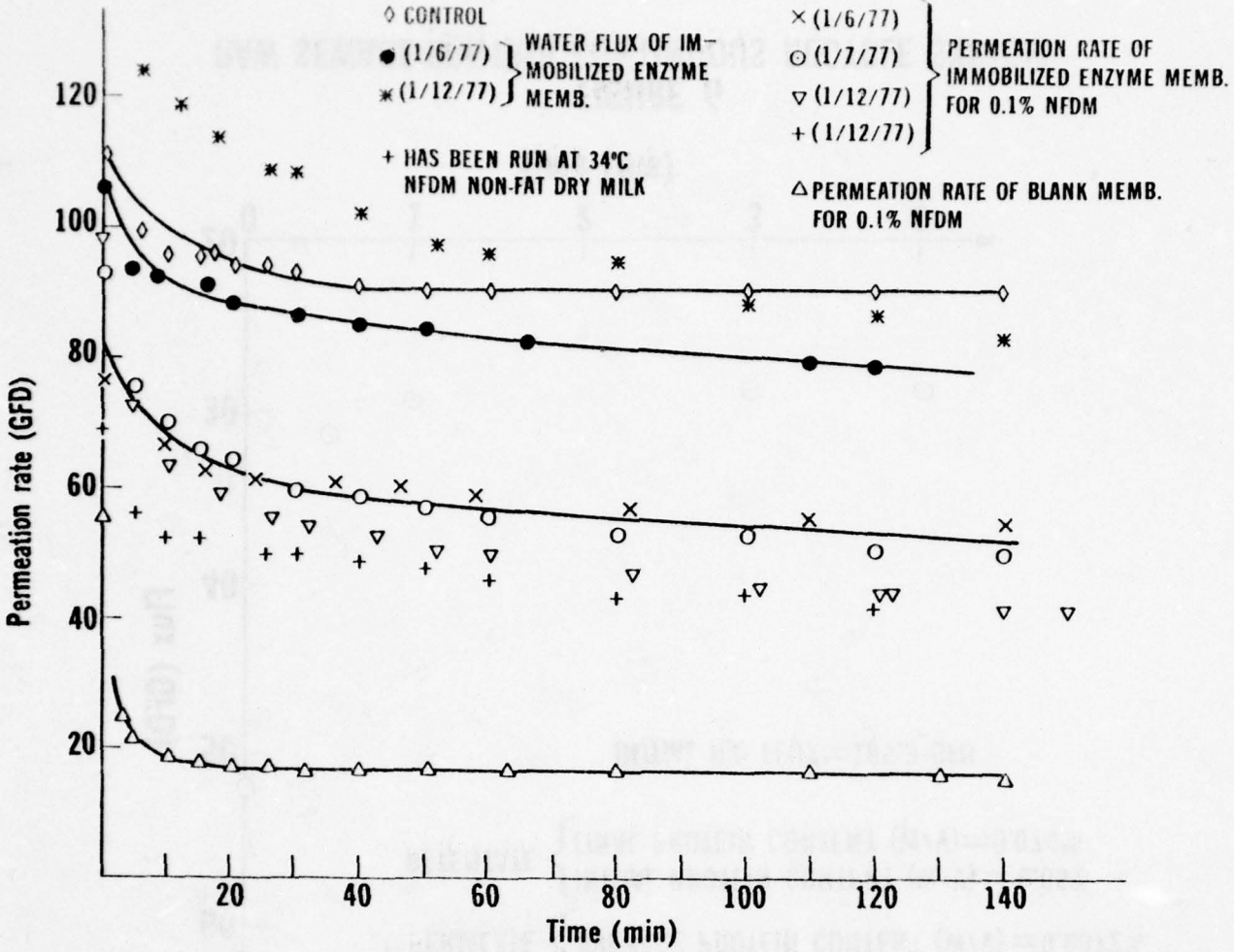


FIGURE 5
THE EFFECT OF RHOZYME B-6 ON THE PERMEATION RATE FOR 0.1% NFDM AT 36°C

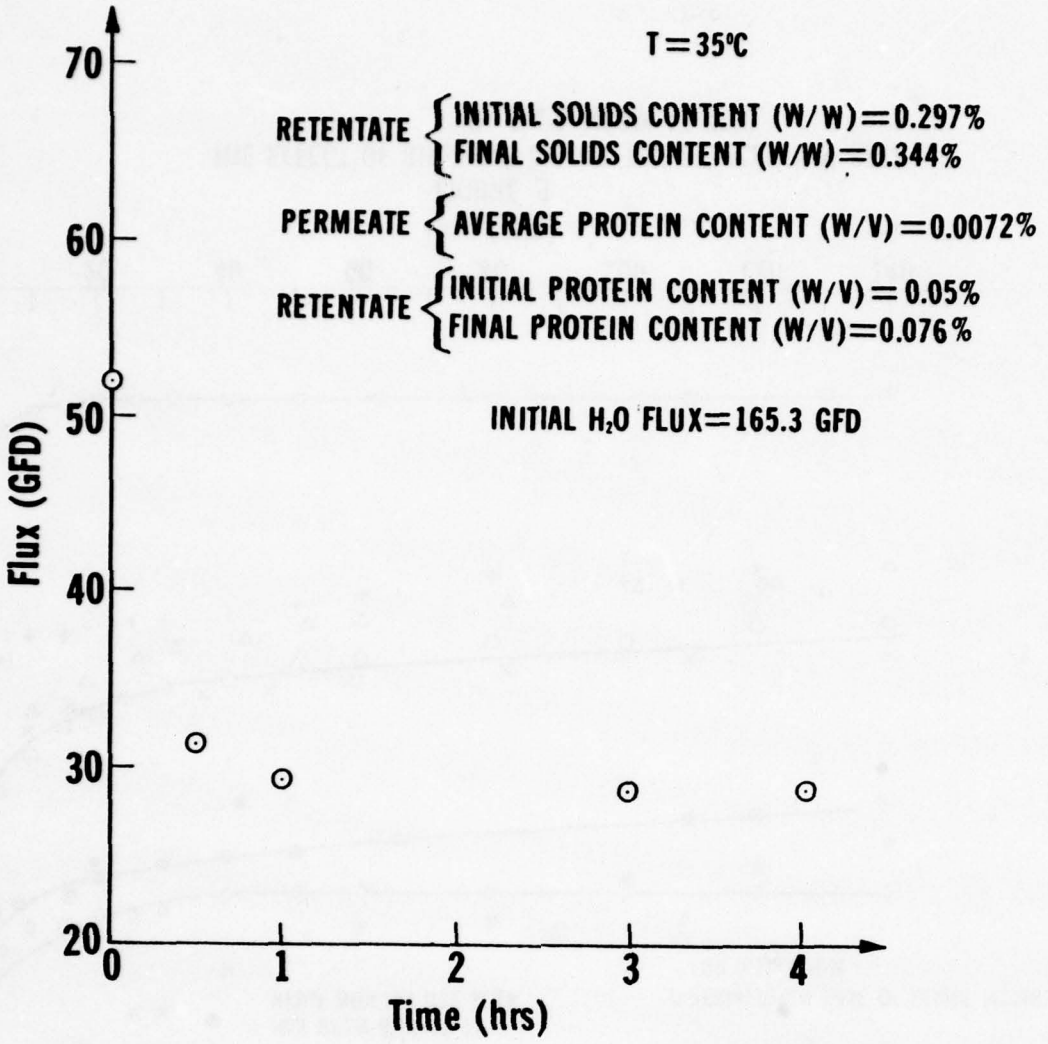


FIGURE 6
RAW SEWAGE-CONTROL-CONTINUOUS RECYCLE SYSTEM

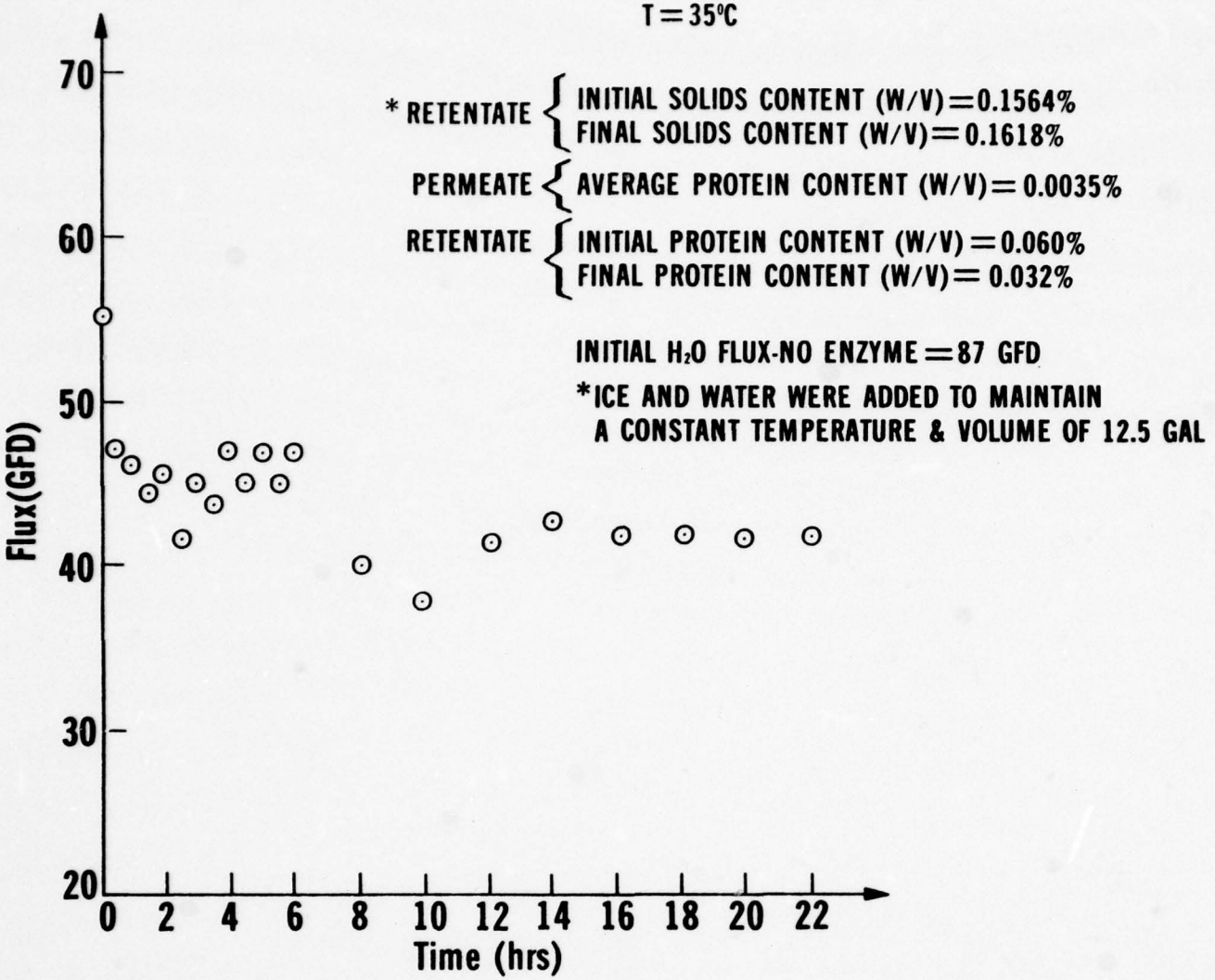


FIGURE 7
RAW SEWAGE-IMMOBILIZED ENZYME-CONTINUOUS RECYCLE SYSTEM

APPENDIX C - INTERIM REPORT (DECEMBER 1976)

RUTGERS, THE STATE UNIVERSITY OF NEW JERSEY
College of Engineering
Bureau of Engineering Research
New Brunswick, New Jersey 08903

(Revised First Annual Progress Report)

AN ENZYME APPROACH TO THE PREVENTION AND REMOVAL OF GELATINOUS FILMS IN RAW-SEWAGE ULTRAFILTRATION SYSTEMS

Contract N00167-76-C-0022

by

Professors Burton Davidson and Shaw S. Wang
Principal Investigators

and

Professor Feng-Chi Hsieh, Assistant Research Professor

Submitted to:

Lynne R. Harris
Department of the Navy
David W. Taylor Naval Ship Research and Development Center
Annapolis Laboratory
Annapolis, Maryland 21402

GENERAL CONSIDERATION AND DESCRIPTION OF THE STATUS OF THE PROJECT.

The amount of enzyme immobilized on the membrane is dependent on the method of immobilization. The direct adsorption or complexation of enzymes onto the membrane depends on the "driving force" of the enzyme molecules (concentration, pressure, etc.) in the solution and molecular diffusivity. High concentration of enzyme solution, high pressure exerted on the immobilization chamber, and/or vacuum created in the membrane are conditions that facilitate the transfer of the enzyme molecules into the membrane to be adsorbed or complexed by the membrane material. Experiments performed have been designed along these conditions. In a typical experiment, 10%(w/v) solution of Rhozyme B-6 was either showered into a membrane in a vacuum chamber or filtered through a membrane under a pressure of 40 lb/in^2g. Both methods produced immobilized enzyme membranes (IEM) that gave higher fluxes 0.1% casein solution than the control. In most experiments performed, the increase in flux was about 100%. In one experiment, the increment was as high as 350%. The increase in flux is indicative of the action of the immobilized enzyme on the casein solution that possibly led to the prevention or slowing down of the buildup of the gelatinous layer on the membrane. To test this theory, the following analyses were performed.

The concentrations of proteins (Lowry method) in the permeates of the IEM are always higher than that of the blank membrane. This is due to the action of the immobilized enzyme on the casein solution as it is being filtered through the membrane. This membrane has a molecular weight cutoff of approximately 20,000. A preparation of 0.1% casein solution contains about 200 mg/l of proteins that have a molecular weight smaller than 20,000 (data obtained by filtering 200 ml of 0.1% casein solution through a blank membrane). When IEM was used, the protein concentration in the filtrate increased about 30% in several different experiments.

This increase is due to the hydrolysis of casein into polypeptides that have molecular weight smaller than 20,000. This action of the immobilized enzyme, in the meantime, prevented or slowed down the formation of a gelatinous layer that is generally fouling the membrane. The activity of immobilized Rhozyme B-6 was also tested by using Azocoll, a synthetic solid substrate for protease, as the substrate in enzyme assay. Since both enzyme and substrate are both in solid form the reaction rate measured is very slow. However, some activity was demonstrated by the immobilized enzyme after more than 24 hours reaction time.

From these preliminary studies, it is foreseeable that one can design an ultrafiltration unit containing immobilized enzyme to prevent the fouling of the membrane by biological materials. In the treatment of raw sewage, since there is about 50% (w/w) protein in the gelatinous layer built up on the membrane, it occurred to use a potent protease to prevent membrane fouling. We have selected Rhozyme B-6, a bacterial protease commercialized by Rohm and Haas. So far, we have demonstrated the feasibility of using IEM for filtering 0.1% casein solution. In the following, we list the experiments to be done toward the testing and design of using IEM for filtering raw sewage:

1. Optimization of the immobilization technique. Vary the concentration of enzyme solution used for immobilization.
2. Immobilization of enzyme onto a 5-foot membrane cartridge.
3. Use of the immobilized enzyme cartridge for the filtration of
   a. 0.1% solution of dried skim milk
      - test flux.
      - test protein concentration in the permeate.
   b. 0.1% raw sewage
      - test flux.
      - test protein concentration in the permeate.

THE SELECTION OF ENZYMES TO BE IMMOBILIZED ON THE ULTRAFILTRATION MEMBRANE.

The fouling of the membrane used in the ultrafiltration of raw sewage is due mainly to the development of a gelatinous layer on the membrane during the process. This gelatinous layer is mainly composed of proteinaceous material. Precipitation or the agglomeration of this gelatinous material on the membrane is possibly due to the conjugation of soluble protein molecules among themselves (becoming less soluble or insoluble in sewage) or with other materials such as cholesterol, fat, or carbohydrate molecules. The formation of precipitants or conjugated products to some extent depends on the size of the precursors. Other things being equal such as hydrophobe/hydrophile ratio , the larger the molecule the smaller is its solubility. This phenomenon can be illustrated by the haze developments in unclarified beer (draft beer) or apple juice during storage. The development of haze in the bottled unclarified beer is due to the aggregation of soluble proteins to form larger, insoluble materials. In the beer industry, it is a general practice that papain, a protease from papaya, is used to hydrolyze these potentially troublesome soluble proteins into smaller polypeptide molecules to prevent the development of haze of bottled beer during storage. One of the faculty members at Rutgers, Dr. S. S. Wang, worked on the clarification of beer by immobilized papain on collagen and found the treatment to be successful. The results were also confirmed by a midwest brewery. The application of that technology in beer clarification now is a matter of economics and government regulation. We feel that in ultrafiltration of raw sewage a similar approach can be applied to prevent the formation of large molecular weight material that will precipitate on the membrane. In searching for a potent enzyme, we have tested the following commercially available industrial enzymes:

1. Rhozyme H-39: Rohm and Haas, Supplier.
2. Rhozyme CL: " " "
3. Rhozyme B-6: " " "
4. Sanizyme: Miles Laboratories, Supplier.
5. KSTUV: " " "
6. Compound C: " " "

We decided on Rhozyme B-6, mainly a potent protease source, to test our hypothesis of using immobilized enzyme for the alleviation of membrane-fouling problem in the ultrafiltration of biological materials. The technique and results obtained from the studies with Rhozyme B-6 can be applied easily to other enzymes.

THE SELECTION OF IMMOBILIZATION METHOD.

Immobilization of enzymes has been one of the interesting developments in the field of Biochemical Engineering in the last 6 years. This field of research drew specialists and experts from different fields (such as biochemistry, microbiology, food science, and chemical engineering). With the efforts put forth by these researchers and those efforts that came from industrial applications, the field of "Enzyme Engineering" caught the eyes of the professionals and evolved as a unique subject within the framework of chemical and/or biochemical engineering.

As an "Enzyme Engineer" one considers both technical feasibilities (methods of immobilization and design of reactors housing the immobilized enzymes) and economical feasibility of a process using enzymes as catalysts.

If the economical factor is an important determinant in the feasibility studies, one would select the cheapest available enzymes and the easiest (and usually the cheapest) method of immobilization. One can basically categorize the various methods of immobilization of enzymes into three groups:

1. Chemically activated covalent bond forming method: cyanogen bromide method, triazine method, etc.
2. Secondary bond/bonds-forming method: collagen complexation, adsorption onto organic or inorganic supporting materials.
3. Physical entrapments: enclosed in hollow fibers, in acrylamide gel, etc.

Methods in group 1, in general, cost more to perform than those of groups 2 and 3. The methods included in group 2 are easy to perform, and in most cases recharge or reimmobilization of active enzyme onto the support is feasible and relatively simple. This would give one a great economical advantage if the supporting material does not only serve as matrix for the enzyme in the process, but also serves in other functions such as ultrafiltration membrane. The use of enzymes for the possible alleviation of membrane fouling in the ultrafiltration of raw sewage or food materials, such as whey, falls into this category. Because of such reasoning, we decided as the first priority to use the adsorption method for the immobilization of enzyme/enzymes onto the ultrafiltration membrane.

EXPERIMENTAL AND RESULTS

1. Experimental Setup. All enzyme screening tests were conducted in a batch process. The sketch of the apparatus is shown in Figure 1. Raw sewage samples of ca. 0.1% suspended-solids solution, were added to the ultrafiltration module and then ultrafiltered under a pressure of 40 lb/in^2g. During operation the solution inside the module was stirred by a suspended magnetic stirrer. The permeation rate was recorded as a function of time.

2. Static Test. This is the first experiment of the enzyme screening tests. Raw (20 ml) sewage was added to a 50 ml of Amicon Draflo apparatus. Enzymes were added directly to the sewage before filtering. The solution was then forced through the membrane. When 15 ml of permeate had been collected, the experiment was stopped.

The average flux was calculated. Both the permeate and retentate were analyzed for protein and fat content. The method used for the total protein determination was taken from "Protein Measurement with the Folin Phenol Reagent," by Lowry et al, The Journal of Biological Chemistry (Vol. 1935, pp. 265-275; 1951). The total fat-analysis method was described by Van de Kamer et al, in "Rapid Method for the Determination of Fat in Feces," in the same journal (Vol. 177, pp. 347-355, 1949).

The results are shown in Table 1. These results did not show that the enzymes affect the flux significantly. We felt that the effect of enzyme could be masked in such an experiment. Therefore, we decided to filter raw sewage through nylon cloth before using it for the experiment. (See paragraph 4).

3. Dynamic Test. In these experiments, a 400-ml Amicon Diaflo Apparatus was used. Enzymes were added directly to 200 ml of sewage in the module. The experiment was stopped when half of the original sewage had passed through the membrane. The permeate fluxes at 3, 30, and 60 minutes were recorded. Both the crude enzymes: Sanizyme, Rhozyme (obtained from Rohm & Haas Co.), KSTUV, Compound C and Bacteria Culture S (obtained from Miles Co.), and the pure enzymes (cellulase, lipase, trypsin, chymotrypsin, and papain) were tested. Finally, the total protein content and total fat content both in the permeate and on the membrane were analyzed. The total suspended solids were also measured in the permeate.

A Cleaning agent, Triton (obtained from Rohm & Haas Co.), was also tested. The results are shown in Table 2. Sanizyme and lipase show improvement in permeation rate.

The above experiments reveal that the permeation rate in an ultrafiltration process depends strongly upon the solid content. In the batch system, the solids plug the membrane surface in a short period of time. The effect of enzymes, therefore, can be told only in the beginning of the operation in the batch system.

The next experiment attempted to test the effectiveness of enzymes to destroy the gelatinous layer. The procedures were:

a. Raw sewage (200 ml) was filtered through an uncleaned membrane.

b. The membrane was removed and cleaned physically before the filtration was repeated with a second raw sewage sample.

c. This membrane was then soaked in an enzyme solution for about 36 hours before a third raw sewage sample was filtered.

d. The fluxes found in the three filtration runs were compared.

The results are shown in Table 3, and Figure 3 a-e, which indicate lipase, sanizyme, and Rhozyme B-6 were capable of destroying the gelatinous layer. However, inspection of Table IV, and Figure 4 a-f, revealed that combinations of lipase with Sanizyme or with Rhozyme B-6 decreased the effectiveness of the membrane cleaning significantly. At this time, it is thought that lipase either binds with the proteases or is attacked by the protease. In either case, the desired enzymatic activity would be diminished.

4. Multiple Stages Separation Process. Since the filtration rate strongly depends on the total amount of suspended solids in the sewage, a multiple filtration process was carried out. In these experiments, an optimal amount of solids was removed in every stage so that the filtration rate at every stage of the process was high. Several filter cloths (see Table 5) were obtained (from Komline-Sanderson Engineering Corp., N. J.) and tested.

The results of coupling of the filtration cloths with ABCOR HFM membrane are shown in Table 5. We found that if aluminum sulfate ($Al_2(SO_4)_3$) was added to precipitate the ionic particles before filtering through a fine porosity cloth, the flux increased.

5. Immobilization of Enzyme by Adsorption/Complexation Method. Two techniques, the pressure-adsorption method and the vacuum-adsorption method, were employed to achieve immobilization of enzyme.

a. Pressure-Adsorption Method. The experimental setup described in paragraph 1 was used. Rhozyme B-6 solution, 200 to 300 ml (0.5%) was filtered through a Abcor HFM membrane of known/average water flux. Water (400 ml) was then filtered to wash the membrane. This membrane was then ready for experiment, or assay. In between runs, the membrane was soaked in 10 ml of water.

b. Vacuum-Adsorption Method. In order to facilitate the transfer/penetration molecules into the membrane, a vacuum was created in the chamber where immobilization was to take place. Essentially, in this process, enzyme solution was sucked into a vacuum chamber and showered onto the membrane in it. After this, the membrane was left in the enzyme solution for about 20 hours. The soaked membrane was then washed with water and used for testing. Successful immobilization through this technique results in a brown membrane which is colored by the enzyme solution. Without proper vacuum this procedure produces membrane which is not well-colored and, hence, has less enzyme immobilized as tested by its enzymatic activity.

6. The Assay of Immobilized Rhozyme B-6. Rhozyme B-6 is mainly a protease. The assay of a general protease can be performed by using different synthetic substrates such as BEAE, TAME Azocoll, etc. This type of assay enables one to follow the catalyzed reaction spectrophotometrically. For convenience, we picked Azocoll^{R} as substrate. The activity of Rhozyme B-6 is followed by the dissolution of a red dye from solid insoluble Azocoll particles (50-100 mesh). It is convenient to assay soluble protease with Azocoll^{R} as substrate. However for immobilized protease, soluble substrates such as BEAE or TAME are better substrates because of the possible complication of mass transfer effects.

Another method for the assay of a protease is the filtration of the hydrolyzed peptides through a membrane and measurement of the rate of hydrolysis by titration

of protein/total nitrogen in the filtrate. This method is not as efficient, nor as accurate/reproducible as that using synthetic substrate. However, the advantage of it is the use of a real substrate, namely, a protein/proteins, instead of synthetic substrate which only allows the measurement of one type of proteolytic activity. Our experimental set-up of filtration as described in Section A, is ideal for such assay method. A protein solution (i.e., 0.1% casein) containing protein molecules or aggregates of them with "molecular weight" higher than the membrane molecular weight cut-off can be used as substrate. In such a process the enzyme immobilized on the membrane catalyzed the hydrolysis of the protein as the filtration process goes on. In the meantime, hydrolyzed protein with molecular weight smaller than the molecular weight cutoff of the membrane (an average value of 20,000 for ABCOR HFM membrane), passes through the membrane. The protein concentration in the filtrate minus a blank reading is indicative of the activity of the immobilized protease.

7. Characterization of Rhozyme B-6 (free and immobilized) and Optimization of the Immobilization Technique.

a. The activity of free Rhozyme B-6 was also tested by filtration of the enzyme reacted solution through ABCOR HFM membrane using 1.0% casein solution. The specific activity of the free enzyme k_3 = 60 mg of titratable protein formed/min/g enzyme.

b. The activity of immobilized Rhozyme B-6 was tested both by ultrafiltration 0.1% casein solution through the immobilized enzyme membrane (IEM) and by chopping and grinding the IEM into small particles and its enzymatic activity assayed in a stirred reactor.

(1) Filtration Method, data of a typical experiment:

	Protein Conc. in the permeate mg/l
(a) Blank membrane + H_2O	0.00

		Protein Conc in the permeate mg/l
(b)	Blank membrane + casein substrate (0.1%)	236
(c)	Blank membrane + enzyme	255
(d)	Immobilized membrane + casein substrate	326

(e) Total enzyme used: 0.5% in 200 ml, or 1000 mg.

the amount of enzyme immobilized is

1000 mg - 255 mg/l = 1000 mg - 51 mg (200 ml) = 949 mg

(f) Product concentration is 326 -236 = 90 mg/l = 18 mg/200 ml

Specific Activity of the immobilized enzyme

= mg of product formed/min/g enzyme = 18/60/949

= 3.2×10^{-4} mg/min/mg of enzyme = 3.2×10^{-1} mg/min/g of enzyme

or Specific Activity/cm^2 = 3.0×10^{-1} mg/min/πr^2

= 1.69×10^{-2} mg/min/cm^2 membrane.

The enzyme immobilized on the membrane was done by the pressure adsorption method. The small Specific Activity obtained is due to several factors. Two of these are mass transfer resistance, enzyme leaching.

(2) When Azocoll was used as the substrate, the IEM used for filtering 0.1% casein solution was found to be active in releasing the dye from the solid substrate. Based on the dye releasing rate, the activity of the IEM was found to be

$$\frac{0.48 \text{ O.D.}}{60 \text{ min} \times 0.989 \text{ g enzyme}} = 0.0081 \text{ O.D./min/g enzyme}$$

This is much lower than that found for free Rhozyme (about 0.1 O.D./min/g enzyme). Again, the low value of specific activity is possibly due to enzyme leaching, mass transfer resistance, and others.

c. The amount of Rhozyme B-6 immobilized on the membrane can be determined

by titrating the amount of protein on IEM. This can be done by either the Lowry method of protein determination or total nitrogen analysis.

d. Optimization of immobilization technique will be determined by the amount of enzyme immobilized and the flux of substrate through IEM.

8. The Performance of an Immobilized Enzyme Membrane. A typical experiment comparing an immobilized enzyme membrane with a blank membrane:

Rhozyme B-6 was immobilized on a membrane and this membrane was compared to an untreated membrane or control. The water flux was observed first. Then 350 ml 0.1% casein was ultrafiltered until 175 ml permeate was obtained. A 1.6 ml was taken from the permeate for a protein determination. This procedure was repeated twice. Flux and protein concentration in the permeate were compared for both systems.

The membrane immobilization was conducted by the vacuum adsorption method. The membrane was soaked 22 hours in enzyme solution and was kept in an aqueous environment. The results of this experiment are shown on Figures 2, 5, 6, 7, and 8.

The percent improvement of flux for immobilized enzyme membrane over control increases form 26.7% to 64.3% after three volume replacements in the filtration unit. A better comparison method is to measure the area under the flux curves. For three consecutive volume replacements, the areas under the flux curves of the immobilized system were 26%, 200%, and 350% greater than the areas under the control curves (see Figures 2, 5, and 6). Not only was the IEM system better overall than the control system, but the differences between the two appear to increase with each volume replacement and with time. Three sets of experiments performed earlier support the ability of the IEM to significantly improve flux of 0.1% casein solutions. These earlier experiments show that sewage flux is improved in an IEM system.

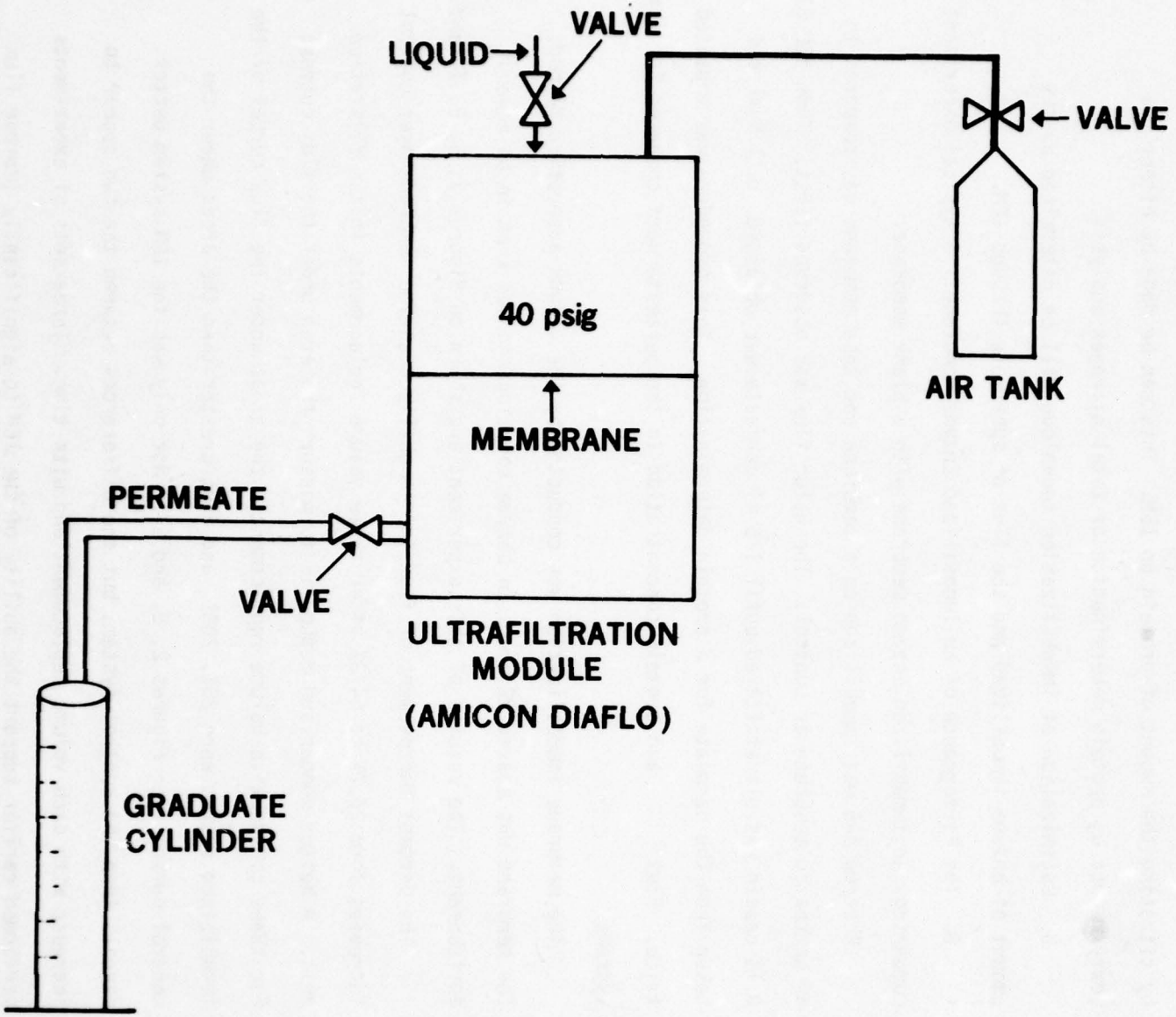


FIGURE 1. SKETCH OF TEST APPARATUS

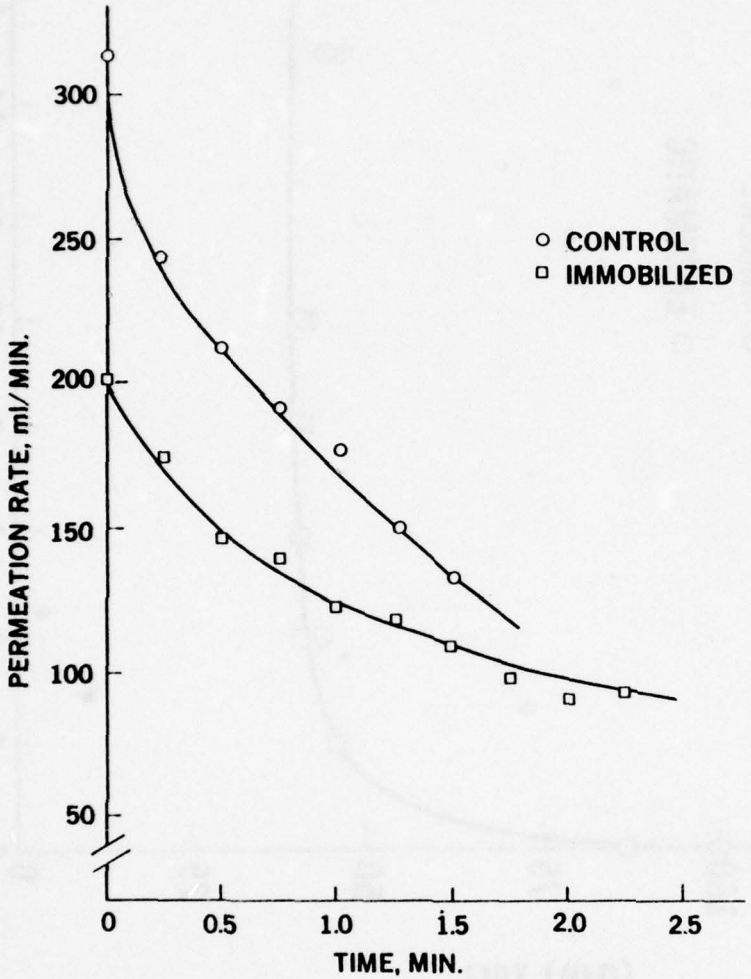


FIGURE 2. PERMEATION RATE OF WATER THROUGH 400 ml AMICON DIAFLO

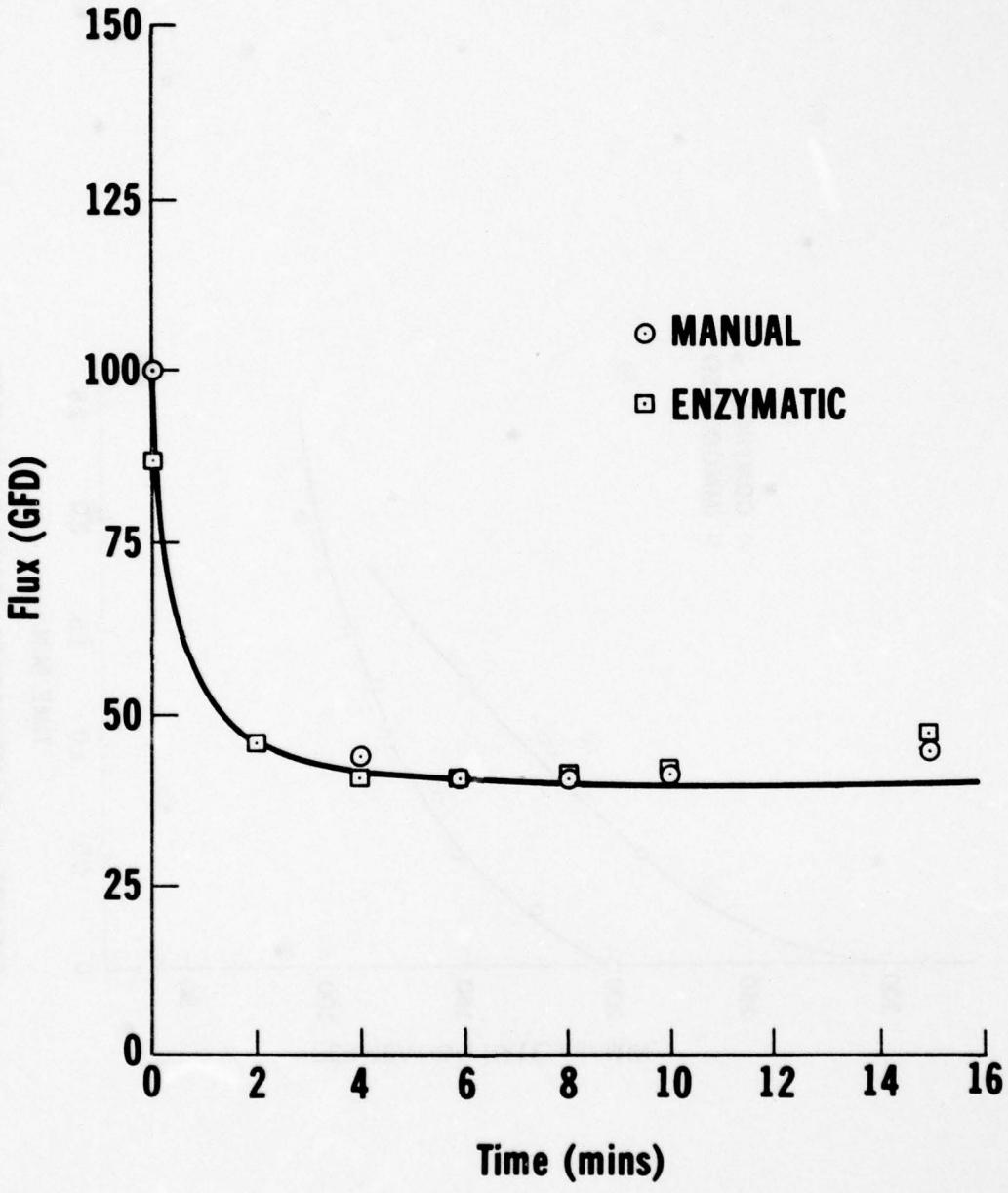


FIGURE 3a

RHOZYME H-39 MANUAL VS. ENZYMATIC MEMBRANE CLEANING
SEPTEMBER 29, 1976

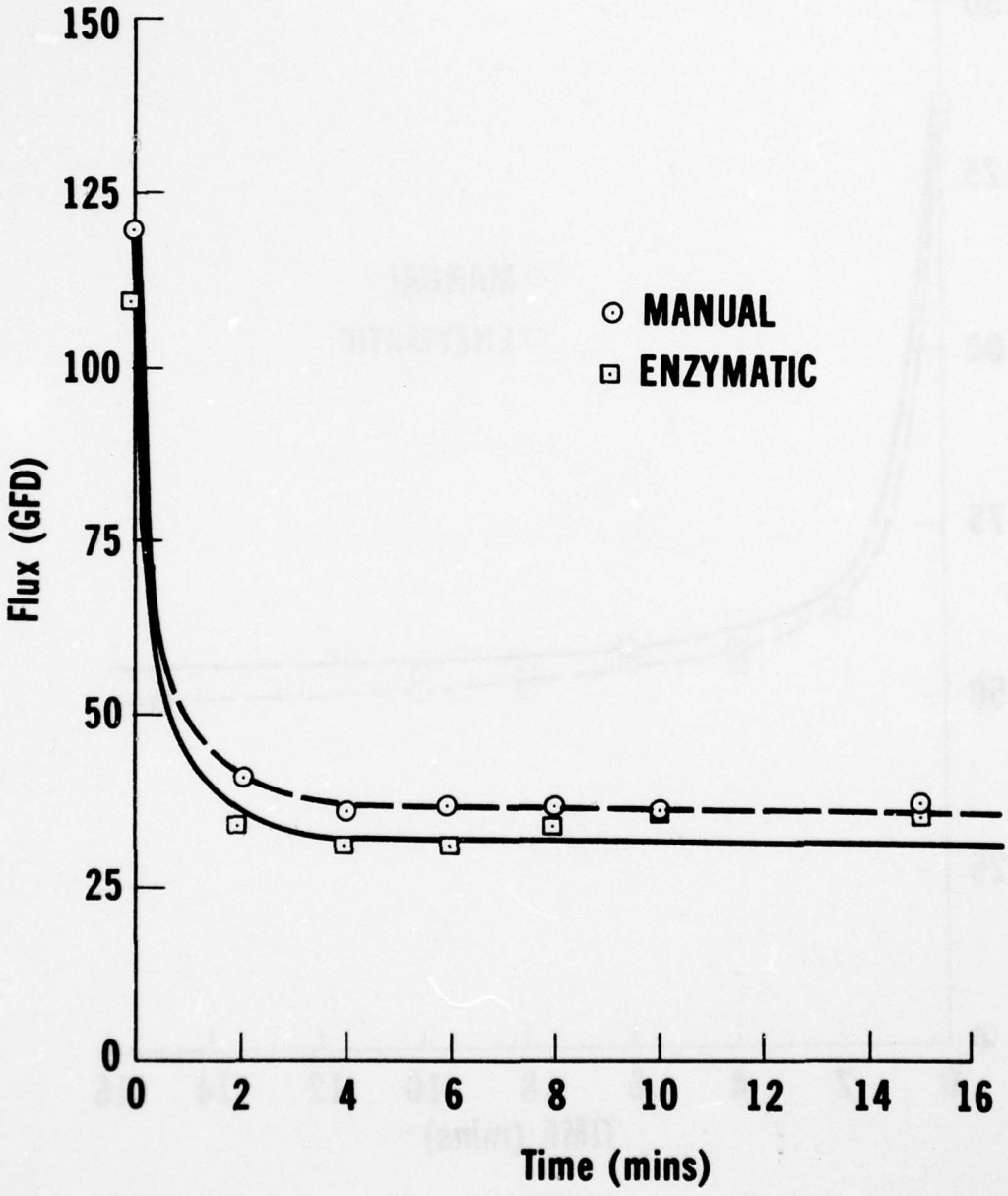


FIGURE 3b
RHOZYME CL MANUAL VS. ENZYMATIC MEMBRANE CLEANING
SEPTEMBER 29, 1976

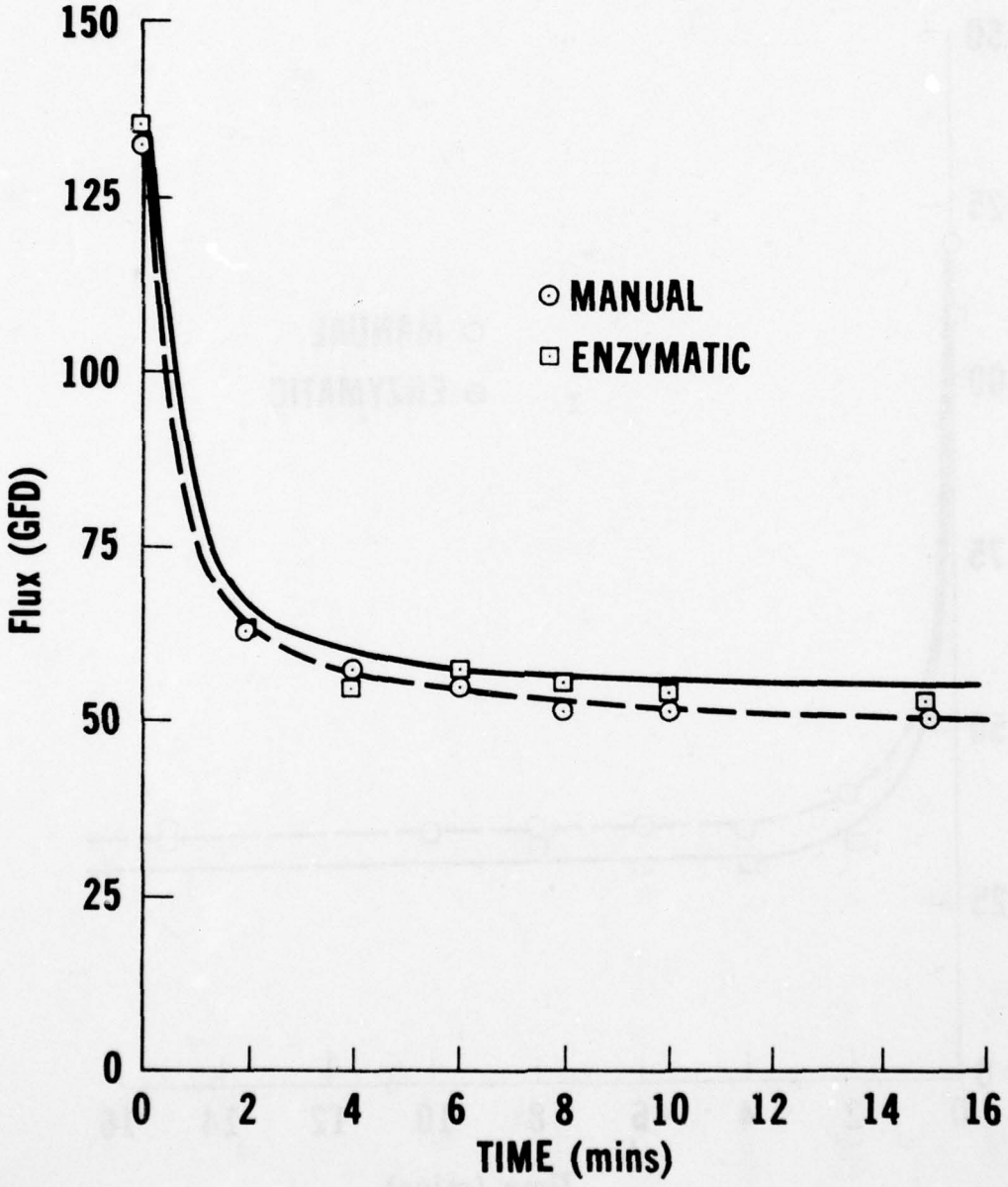


FIGURE 3c
RHOZYME B-6 MANUAL VS. ENZYMATIC MEMBRANE CLEANING
SEPTEMBER 29, 1976

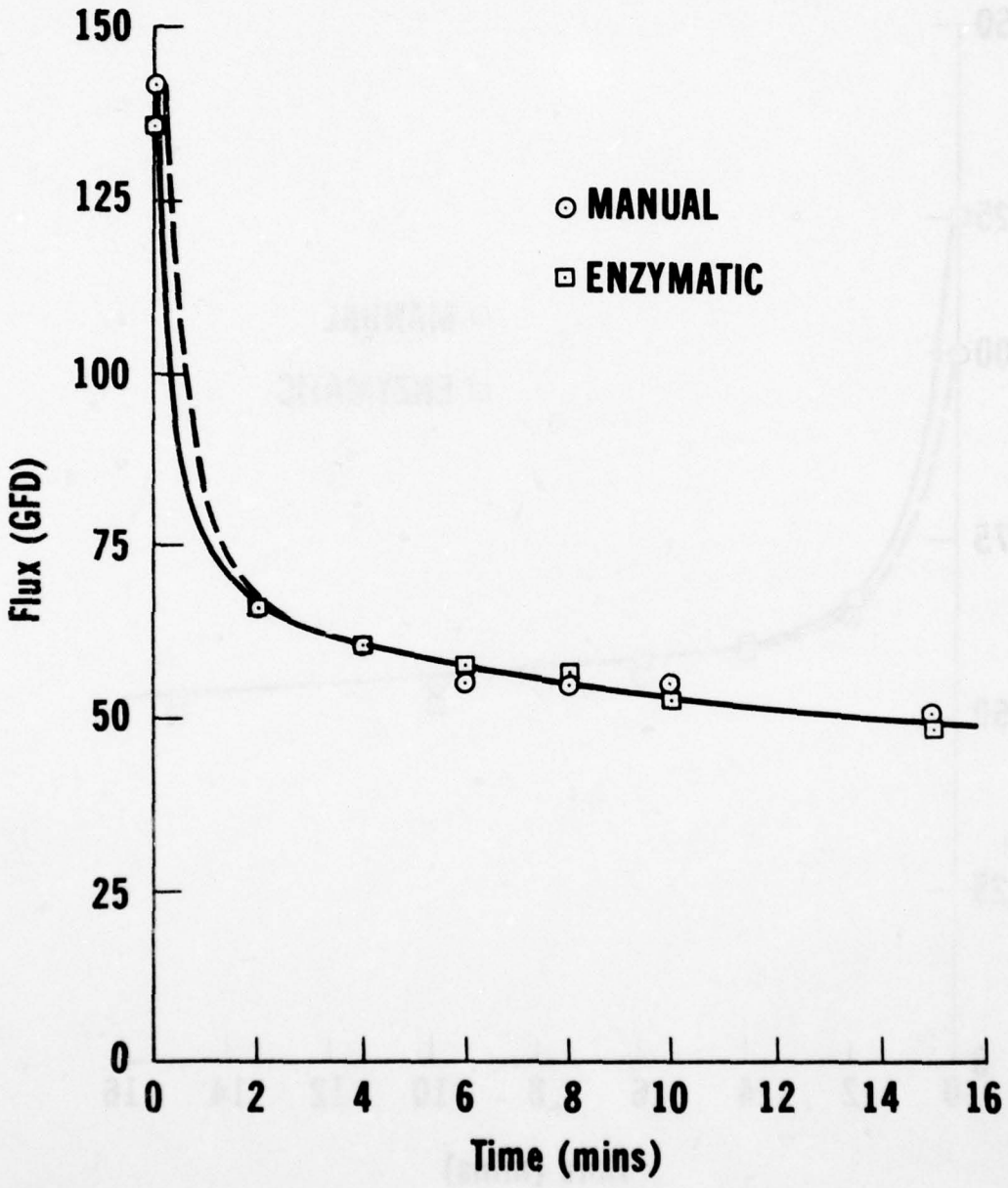


FIGURE 3d

SONIZYME

MANUAL VS. ENZYMATIC MEMBRANE CLEANING

SEPTEMBER 29, 1976

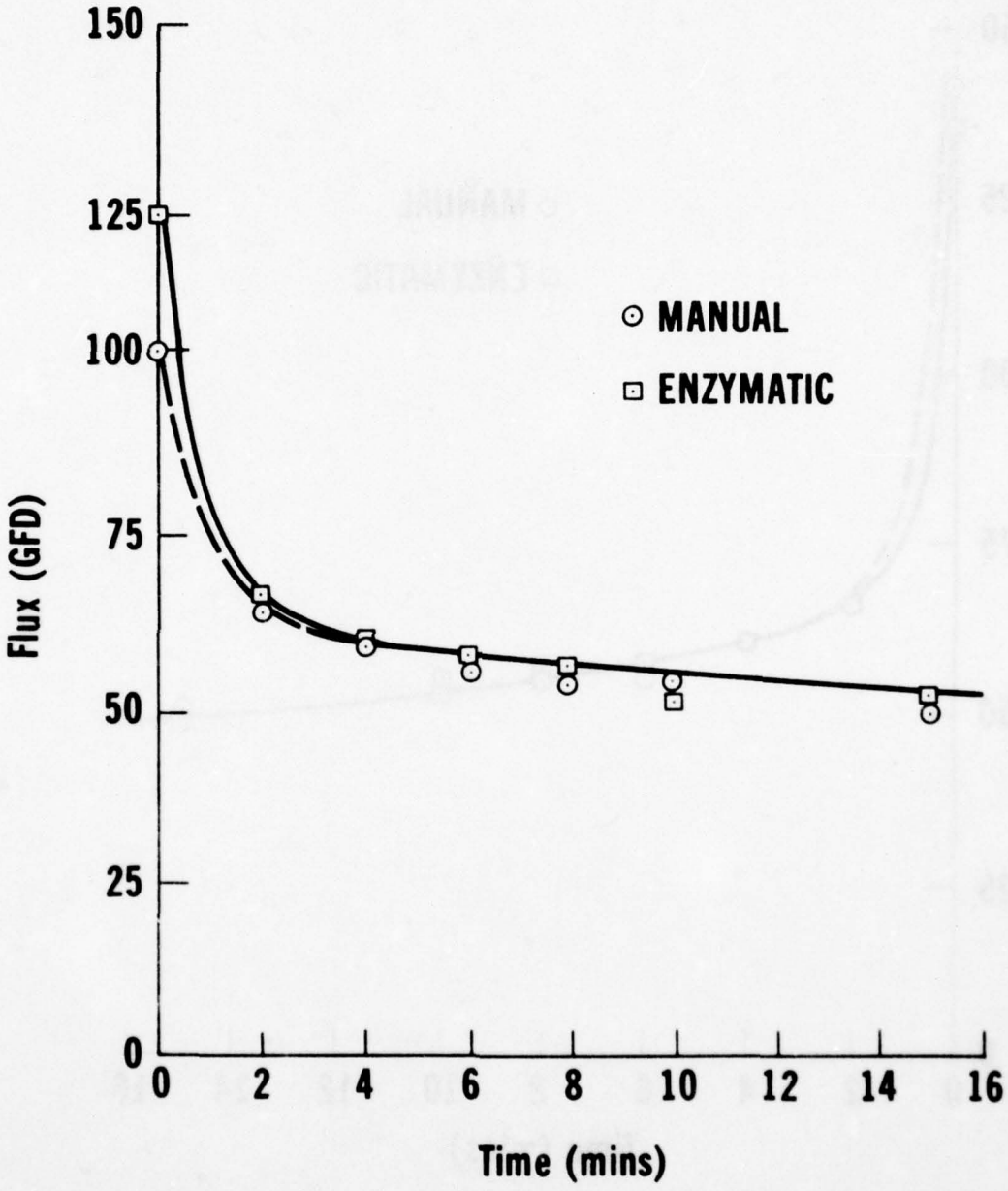


FIGURE 3e
LIPASE MANUAL VS. ENZYMATIC MEMBRANE CLEANING
SEPTEMBER 29, 1976

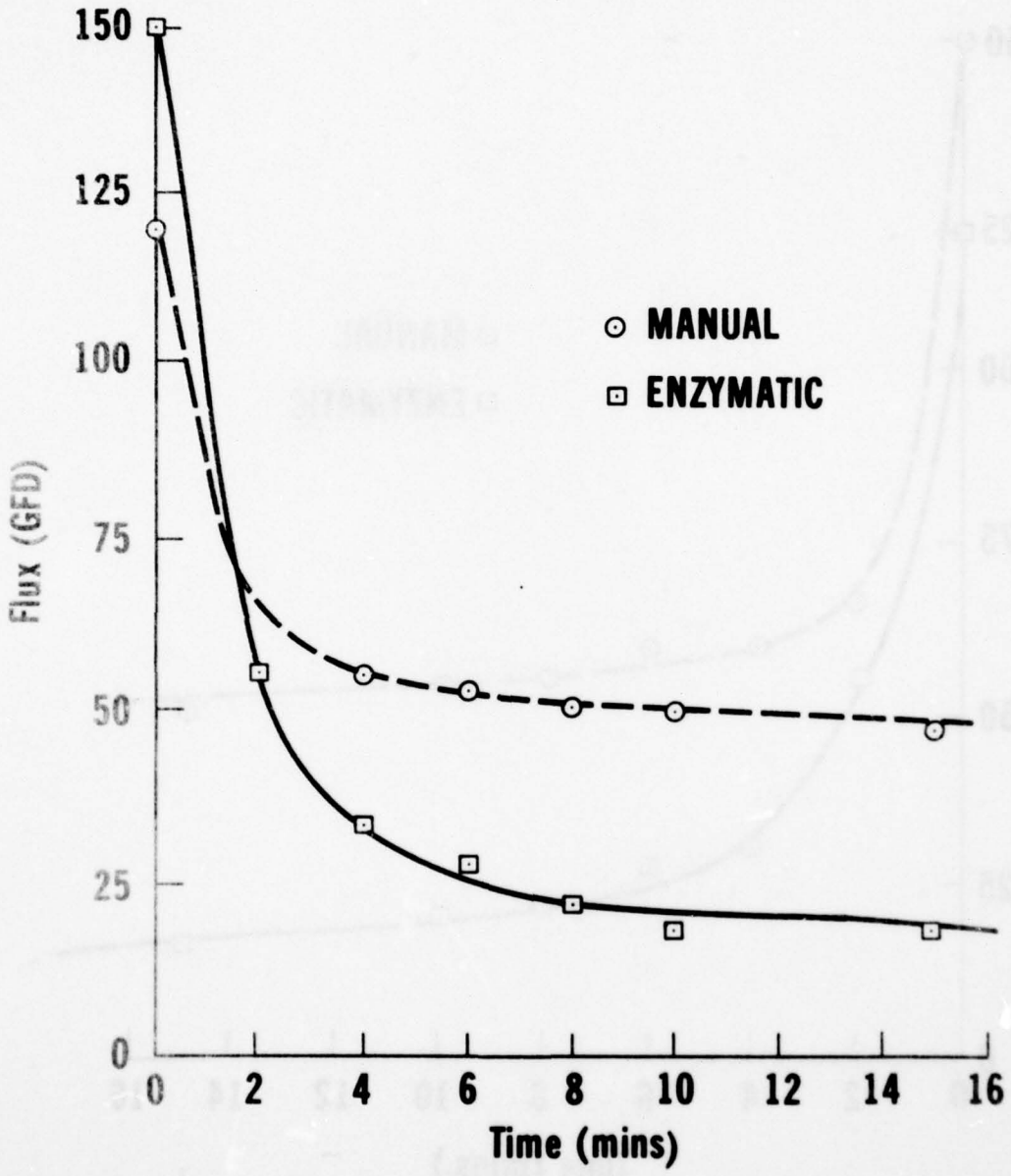


FIGURE 4a
LIPASE
MANUAL VS. ENZYMATIC MEMBRANE CLEANING
OCTOBER 7, 1976

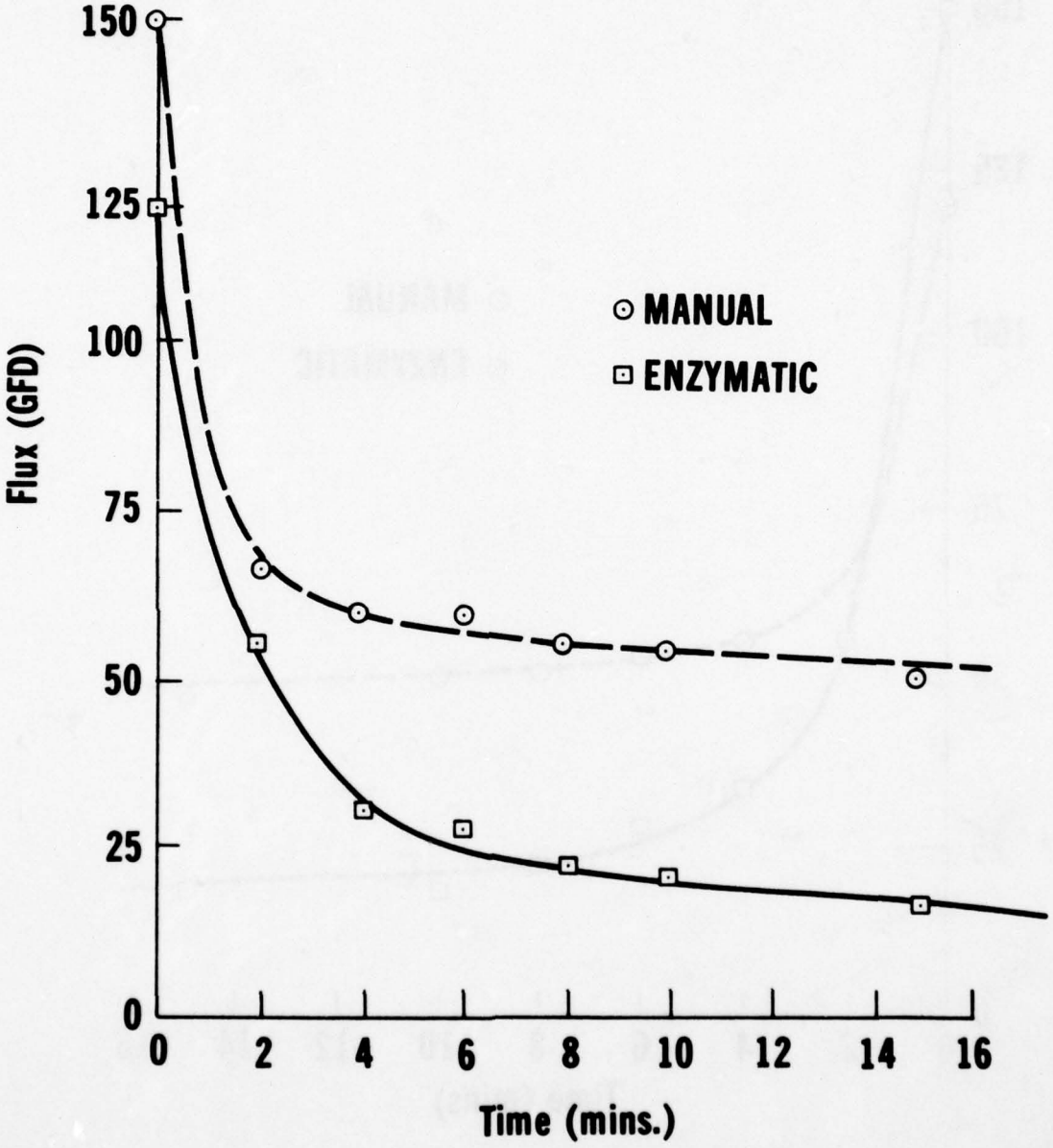


FIGURE 4b
RHOZYME B-6 + LIPASE
MANUAL VS. ENZYMATIC MEMBRANE CLEANING
OCTOBER 7, 1976

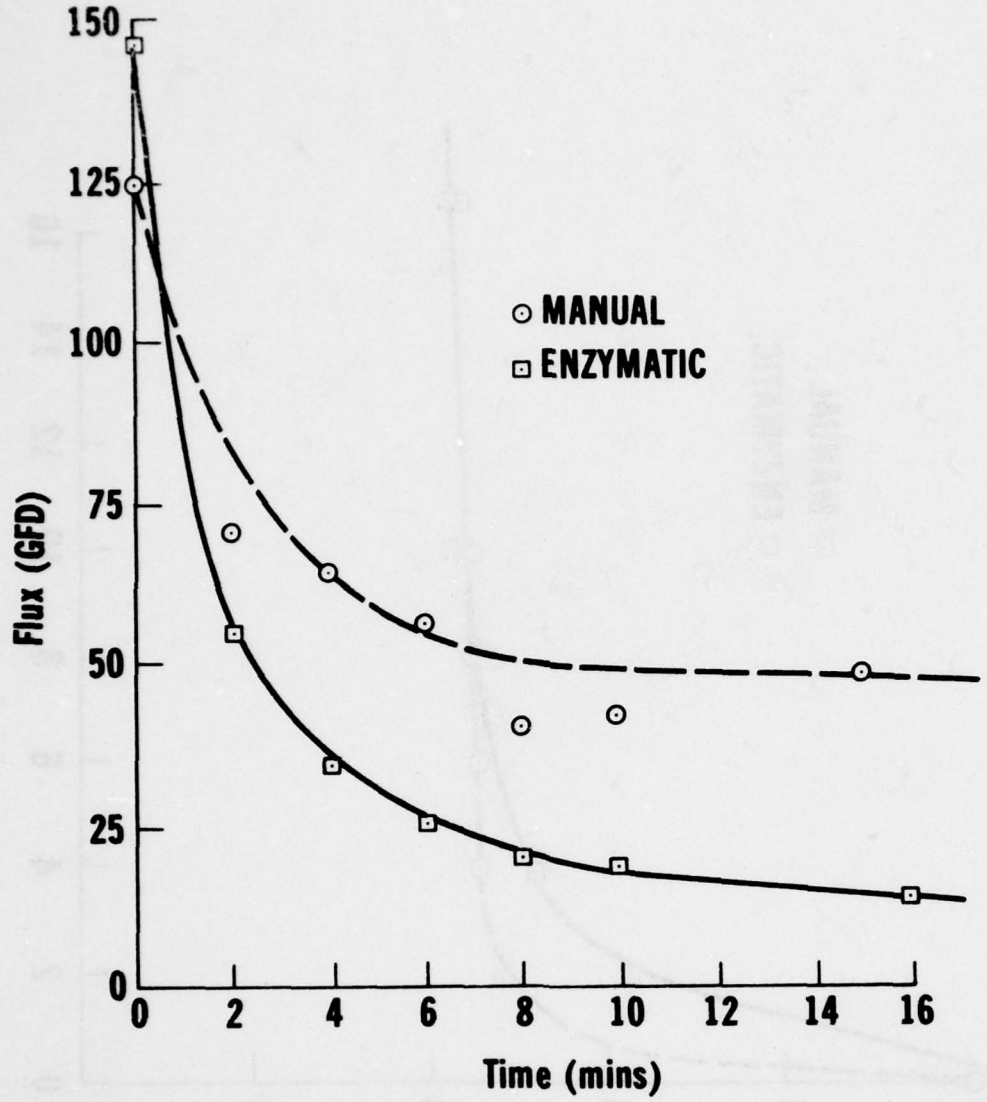


FIGURE 4c
SANIZYME LIPASE MANUAL VS. ENZYMATIC MEMBRANE CLEANING
OCTOBER 7, 1976

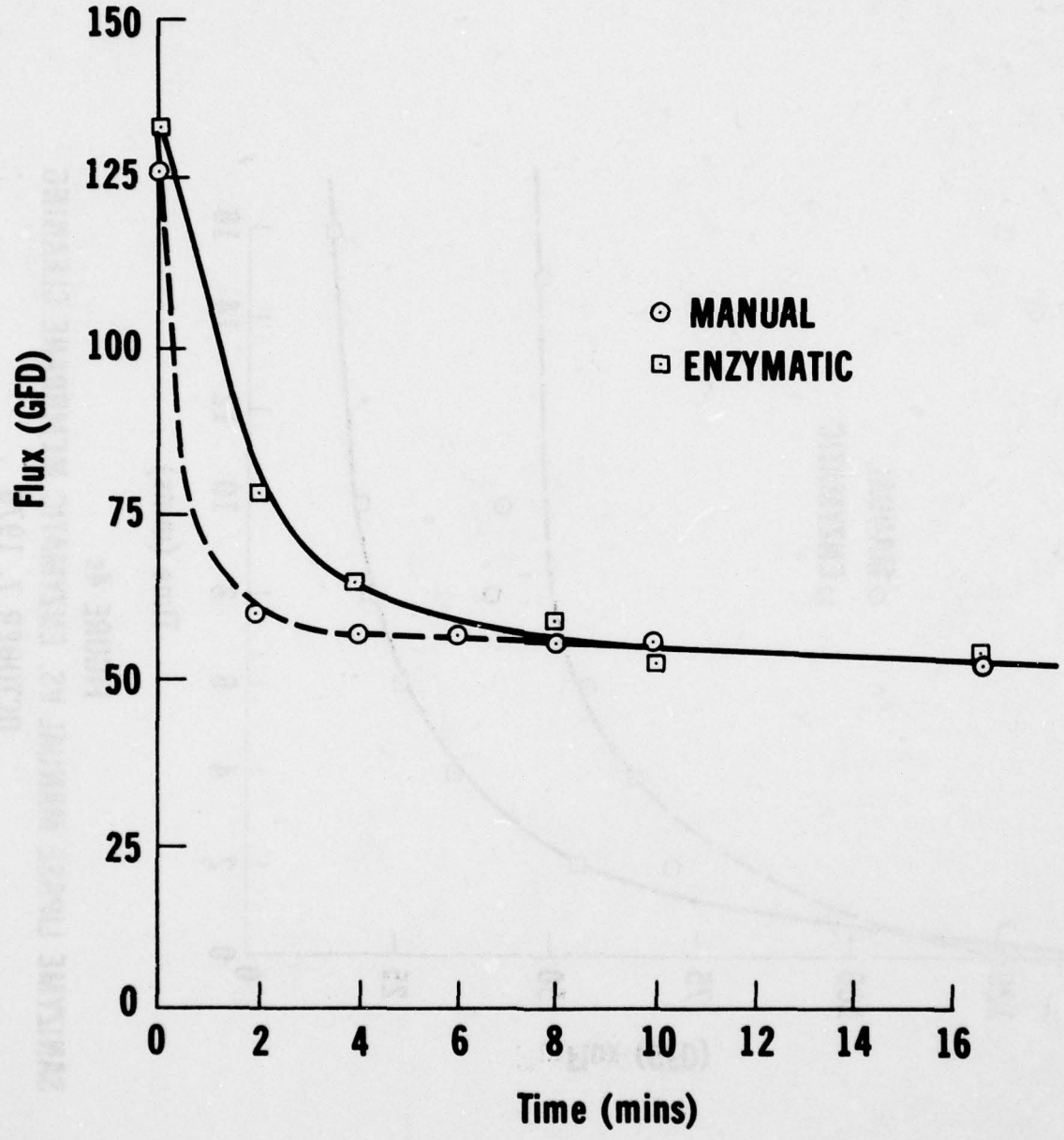


FIGURE 4d
SANIZYME MANUAL VS. ENZYMATIC MEMBRANE CLEANING
OCTOBER 7, 1976

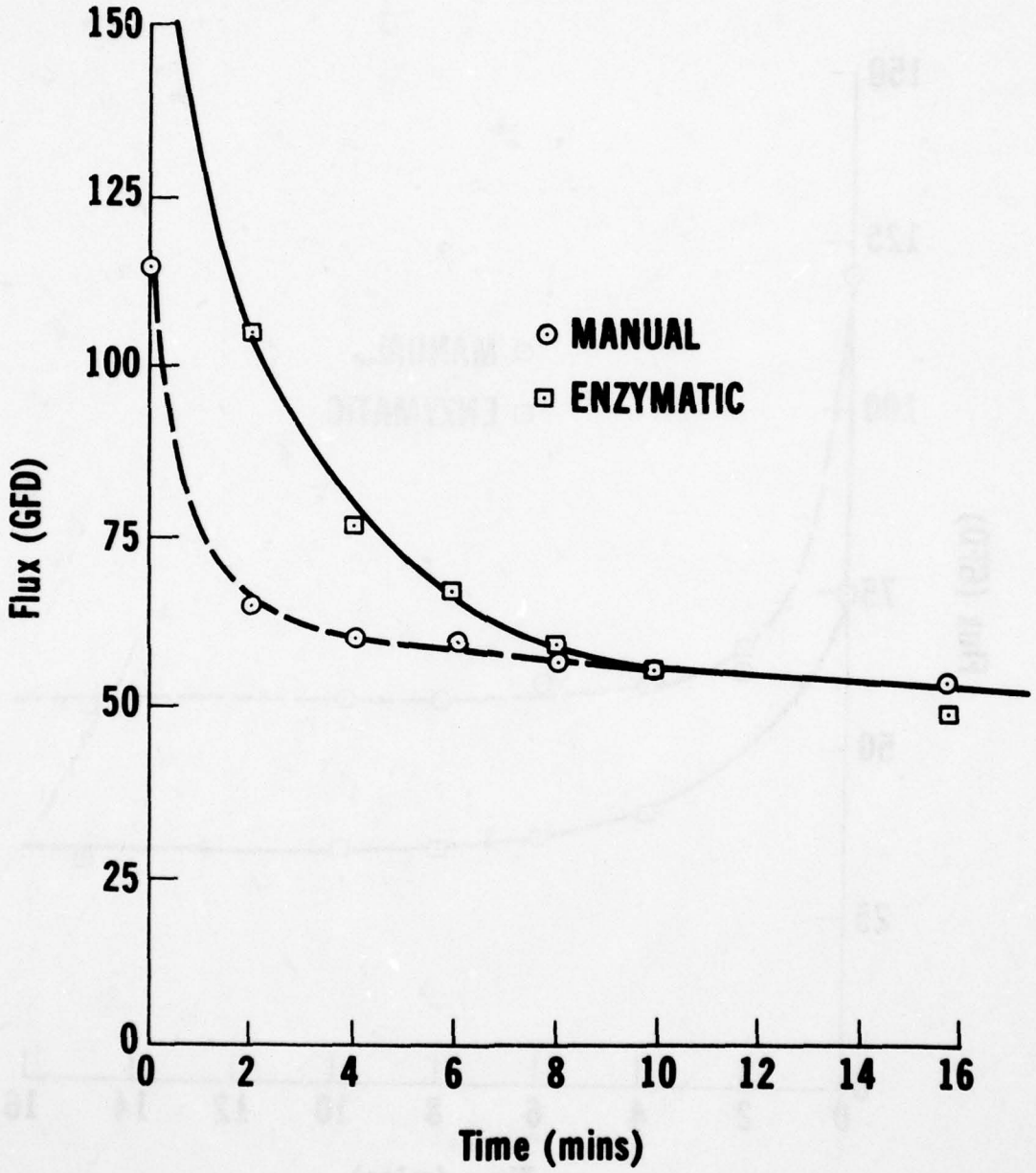


Figure 4e

RHOZYME B-6 MANUAL VS. ENZYMATIC MEMBRANE CLEANING
OCTOBER 7, 1976

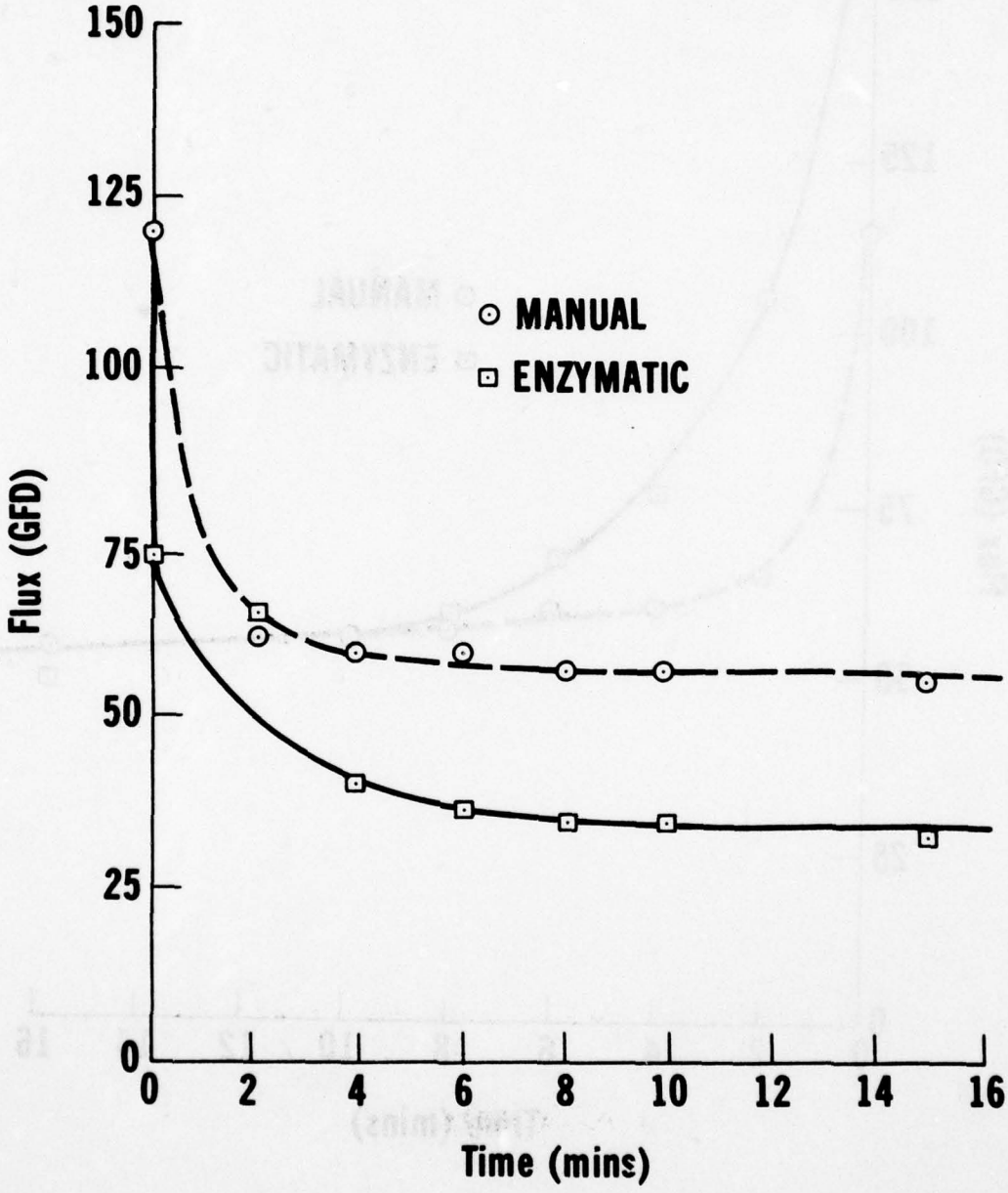


FIGURE 4f
RHOZYME CL MANUAL VS. ENZYMATIC MEMBRANE CLEANING
OCTOBER 7, 1976

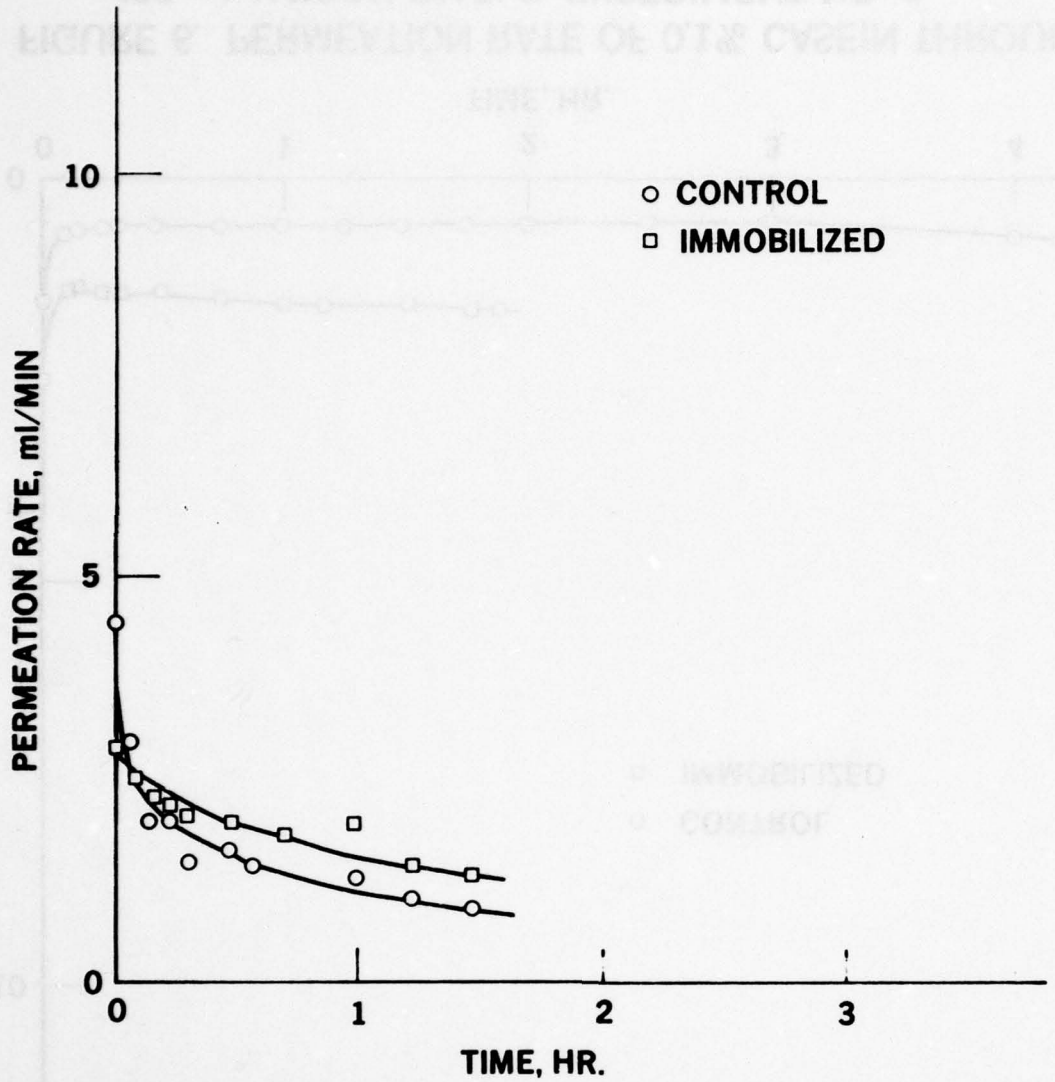


FIGURE 5. PERMEATION RATE OF 0.1% CASEIN THROUGH 400 ml AMICON DIAFLO, EXPERIMENT NO. 1

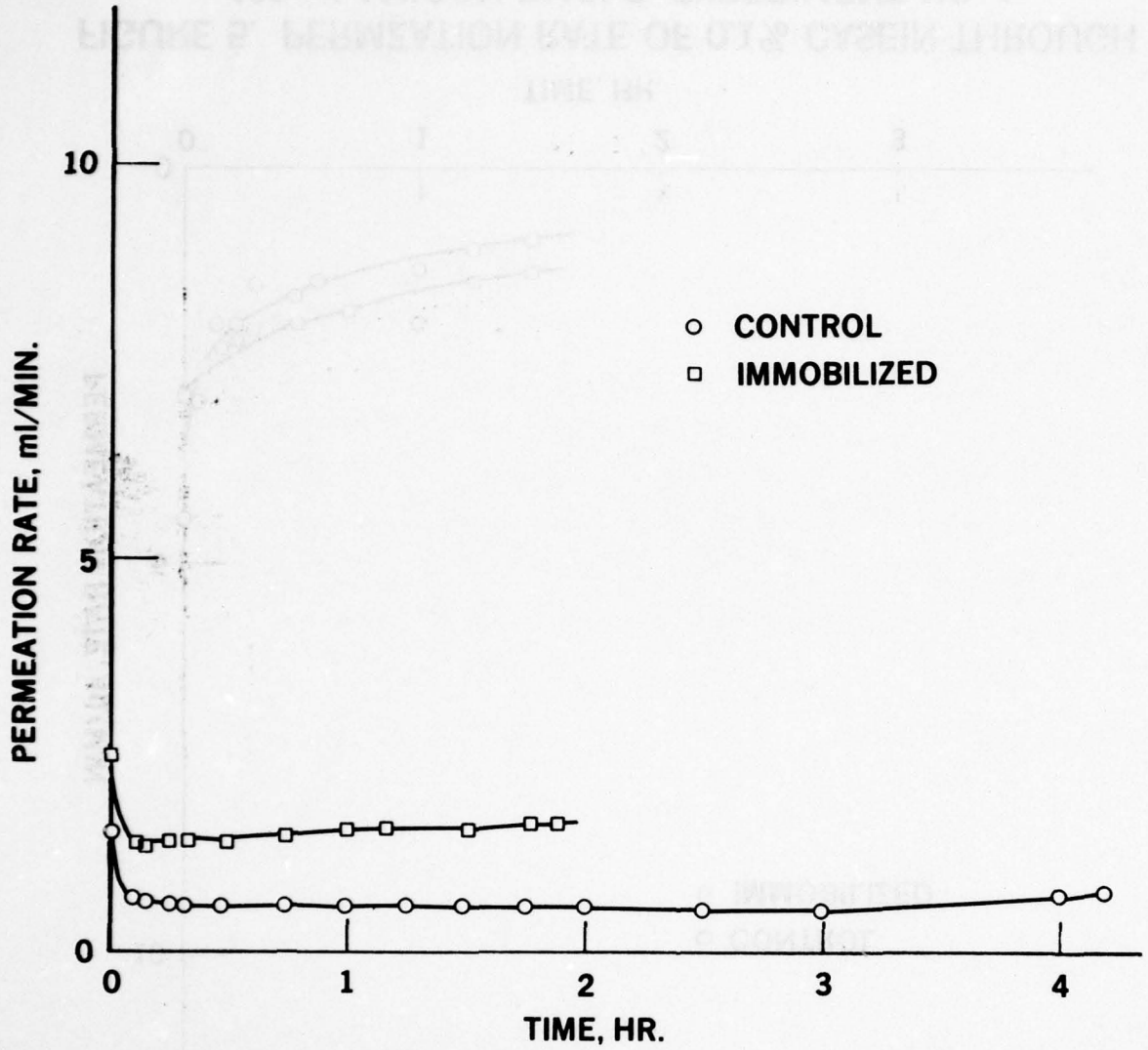


FIGURE 6. PERMEATION RATE OF 0.1% CASEIN THROUGH 400 ml AMICON DIAFLO, EXPERIMENT NO. 2

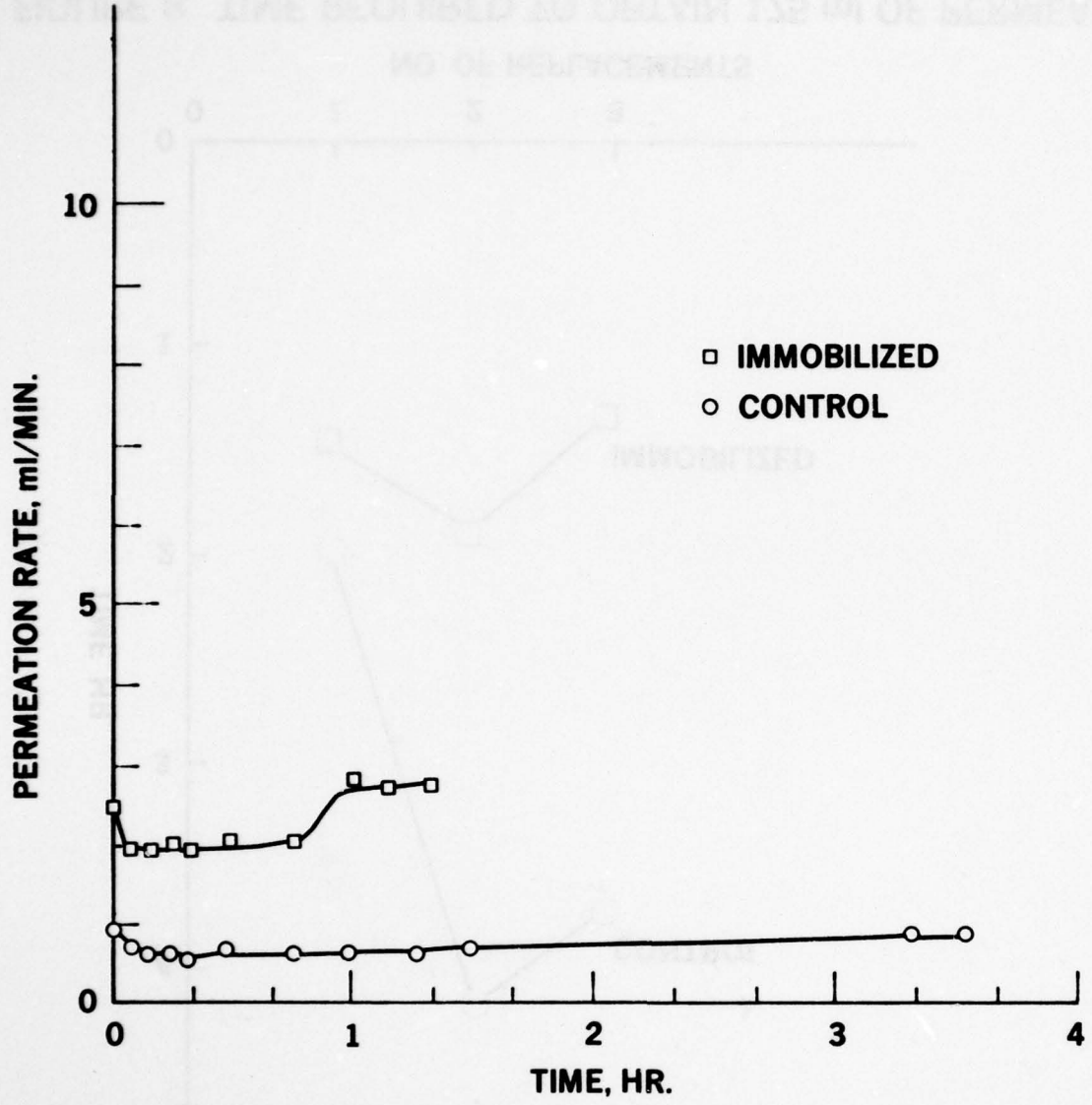


FIGURE 7. PERMEATION RATE OF 0.1% CASEIN THROUGH 400 ml AMICON DIAFLO, EXPERIMENT NO. 3

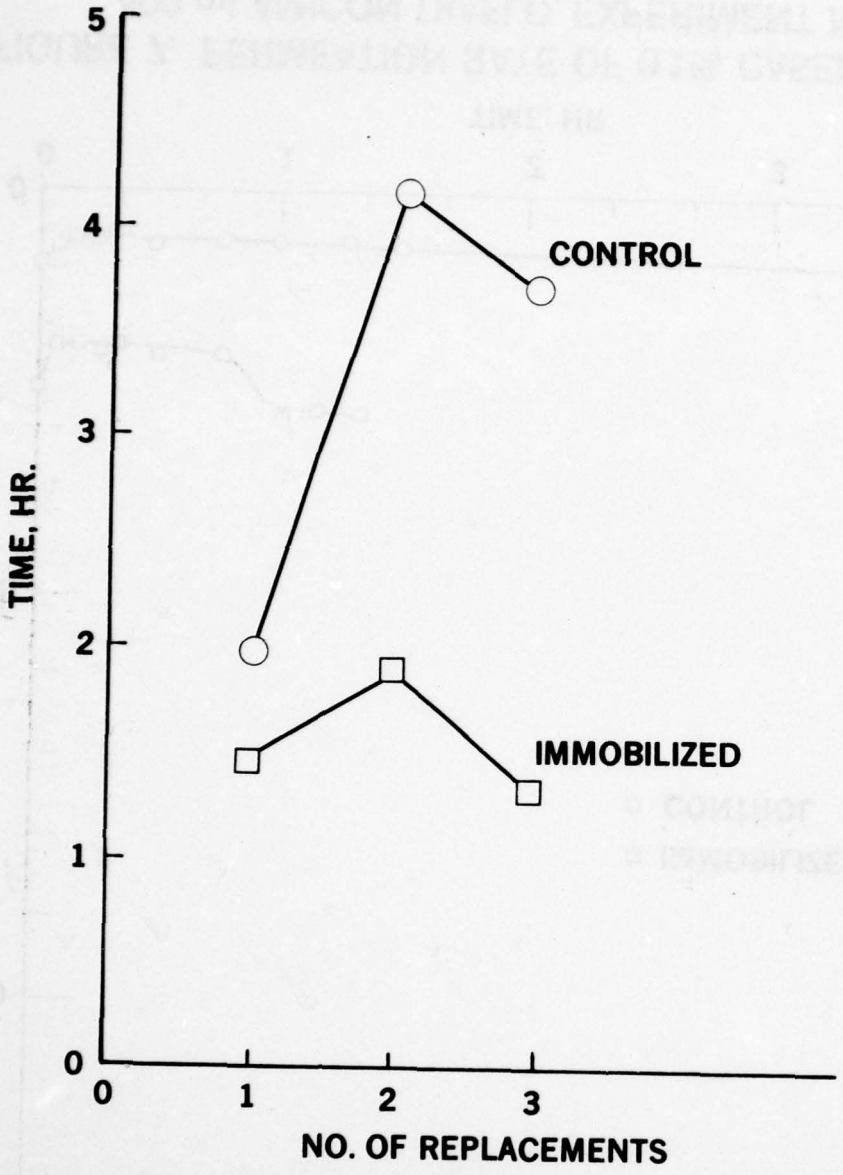


FIGURE 8. TIME REQUIRED TO OBTAIN 175 ml OF PERMEATE VS. NUMBER OF VOLUME REPLACEMENTS

AD-A066 397 RUTGERS - THE STATE UNIV NEW BRUNSWICK N J BUREAU O--ETC F/G 13/2
AN ENZYME APPROACH TO THE PREVENTION AND REMOVAL OF GELATINOUS --ETC(U)
MAR 79 S S WANG, B DAVIDSON, C GILLESPIE N00167-77-C-0001

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Table 1. Static Test (July 1976)

Conditions of Membrane Treatment	Solid Content (mg/ml)	Time to Collect 15 ml (hr)	Average Flux (GFD)	Protein Content Retentate (mg/ml)	Protein Content Permeate (mg/ml)	Fat Content Retentate (mg/ml)	Fat Content Permeate (mg/ml)
No treatment	34.6	6	1.3	241.25	1.05	18.45	0.206
Sanizyme, 0.75 g 44 hr	34.6	2	3.9	38.07	1.2	15.79	0.131
Rhozyme, B-6: 0.3750 g, CL: 0.3375 g, H-39: 0.375 g } 0.75g 30 hr	34.6	3	2.6	455.00	1.7	13.34	0.140

Note: 20 ml of ∿3% suspended-solids solution for each filtration. Ultrafiltration carried out under 40 lb/in^{2}g at room temperature. Effective area of membrane is 1.766 in^2.

Table 2. Dynamic Test (July 30, 1976)

Conditions of Membrane Treatment	Solid Content (mg/ml)	Flux GFD			Protein Content		Fat Content	
		3 min	30 min	1 hr	Retentate (mg/in^2)	Permeate (mg/ml)	Retentate (mg/in^2)	Permeate (mg/ml)
No treatment	0.050	29.5	18.5	18.5	0.2138	0.049	0.0308	0.0035
Sanizyme, 0.75 g	2.308	31.4	19.4	18.5	0.1477	0.154	0.0247	0.0015
Sanizyme, 0.75 g 24 hr	2.056	24.9	11.1	11.1	0.2164	0.092	0.0257	-
KSTUV, 0.75 g	0.428	30.4	15.7	12.9	0.2648	0.198	0.0206	0.0039
Compound C, 0.75 g	-	29.5	16.6	13.8	0.3246	0.309	0.0257	0.0043
Rhozyme, 0.75 g	2.267	24.9	11.1	11.1	0.2927	0.177	0.0236	0.0039
Triton, 0.75 g	0.075	31.4	18.5	18.5	0.1069	0.049	0.0391	0.0029
Lipase, 0.02 g	0.255	39.7	18.5	18.5	0.2189	0.043	0.0308	0.0033
Lipase, papain and triton, 0.75 g	0.426	21.2	12.5	12.0	0.1477	0.045	0.0494	0.0019
Trypsin and chymotrypsin, 0.005 g	0.2155	27.7	12.0	10.0	0.2378	0.053	0.0247	0.0035
Trypsin and chymotrypsin, 0.75 g 20 hr	0.2485	32.3	20.0	10.1	0.3564	0.024	0.0247	0.0010

Note: Enzymes were added directly to the suspended solids solution, 200 ml of ~0.1% suspended-solids solution for each filtration. Ultrafiltration carried out under 40 lb/in^2g at room temperature. Effective area of membrane is 2.75 in 2.

Table 3. Dynamic Test (September 29, 1976)

Conditions of Membrane Treatment	Membrane No.	Flux GFD						
		0 min	2 min	4 min	6 min	8 min	10 min	15 min
None	1	92.2	49.8	45.2	45.2	46.1	41.5	41.5
Cleaned manually	1	100.5	46.1	44.3	41.5	42.4	43.4	45.2
Soaked in 19.3 mg/ml Rhozyme H-39	1	86.7	46.1	40.6	41.5	42.4	43.4	46.1
None	2	102.4	64.6	56.3	51.6	52.6	50.7	47.0
Cleaned manually	2	120.0	41.5	40.6	41.5	41.5	41.5	42.4
Soaked in 19.9 mg/ml Rhozyme CL	2	108.8	34.1	32.3	32.3	35.0	36.0	35.0
None	3	123.6	55.3	49.8	49.8	48.0	48.0	45.2
Cleaned manually	3	132.8	63.6	57.2	55.3	51.6	51.6	50.7
Soaked in 20.9 mg/ml Rhozyme B-6	3	133.7	63.6	56.3	57.2	55.3	53.5	51.6
None	4	133.7	57.2	56.3	54.4	52.6	51.7	47.0
Cleaned manually	4	143.0	66.4	60.0	55.3	55.3	55.3	50.7
Soaked in 21.2 mg/ml Sanizyme	4	137.4	66.4	61.8	58.1	56.3	53.5	48.0
None	5	69.2	55.3	51.6	50.7	52.6	49.8	47.0
Cleaned manually	5	103.3	64.6	59.0	56.3	54.4	54.4	49.8
Soaked in 5.20 mg/ml Lipase	5	123.6	66.4	60.0	58.1	55.3	53.5	51.6

Note: 200 ml of ~0.1% suspended-solids solution for each filtration. Ultrafiltration carried out under 40 lb/in^{2}g at room temperature. Effective area of membrane is 2.75 in2.

Table 4. Dynamic Test (October 7, 1976)

Conditions of Membrane Treatment	Membrane No.	Flux GFD						
		0 min	2 min	4 min	6 min	8 min	10 min	15 min
Cleaned manually	1	116.2	39.7	33.2	32.3	36.0	36.9	36.9
Cleaned manually	1	119.9	48.0	55.3	53.5	50.7	49.8	47.0
Soaked in lipase	1	147.6	55.3	34.1	27.7	23.1	18.4	18.4
Cleaned manually	2	112.5	38.7	28.6	27.7	29.5	32.3	33.2
Cleaned manually	2	148.5	67.3	60.9	59.0	55.3	54.4	49.8
Soaked in lipase + Rhozyme B-6	2	119.9	55.3	30.4	27.7	23.1	21.2	16.6
Cleaned manually	3	182.6	61.8	57.2	55.3	54.4	48.9	48.0
Cleaned manually	3	124.5	71.0	64.6	57.2	40.6	42.4	48.0
Soaked in lipase + Sanizyme	3	147.6	55.3	35.0	25.8	21.2	18.4	14.8
Cleaned manually	4	123.6	60.9	55.3	55.3	54.4	47.0	47.0
Cleaned manually	4	127.3	60.0	57.2	57.2	55.3	55.3	51.7
Soaked in Sanizyme	4	133.7	78.4	64.6	-	57.6	53.5	52.6
Cleaned manually	5	179.9	55.3	57.2	59.0	56.3	58.1	55.3
Cleaned manually	5	115.3	64.6	60.0	59.0	57.2	56.3	54.4
Soaked in Rhozyme B-6	5	189.1	106.1	76.6	67.3	58.1	55.3	48.9
Cleaned manually	6	119.0	57.2	55.3	56.3	58.1	55.3	53.5
Cleaned manually	6	119.0	60.9	59.0	59.0	57.2	57.2	55.3
Soaked in Rhozyme CL	6	73.8	64.6	39.7	36.9	35.0	35.0	33.2

Table 5. Dyanamic Test, Multiple Stages (8/19/76)

Conditions of Treatment, Sequence of Stages			Flux, GFD				
			2 min.	10 min.	20 min	30 min	40 min.
(1)	Control, Single Stage, 40 lb/in^{2}g		36.0	17.5	12.9	11.1	9.2
(2)	(a) K-S No. 210**		25	-	-	8.3	14.8
	(b) ABCOR HFM, 40 lb/in^{2}g		64.6	24.7	18.9	-	-
(3)	(a) K-S No. 201 **	2 lb/in^{2}g		(250 ml of sample passed through)*			
	(b) K-S No. 210	20 lb/in^{2}g		(the filter in 30)			
	(c) ABCOR HFM	40 lb/in^{2}g	44.3	18.4	13.8	12.9	-
(4)	(a) K-S No. 201	2 lb/in^{2}g		Comment Statement			
	(b) K-S No. 531	40 lb/in^{2}g					14.8
	(c) K-S No. 210	2 lb/in^{2}g		Comment Statement			
	(d) Filter paper No. 5050 from Welch Scient. Co,	2 lb/in^{2}g		Comment Statement			
	(e) ABCOR HFM	40 lb/in^{2}g	48.7	28.3	14.8		
(5)	(a) K-S No. 201	2 lb/in^{2}g		Comment Statement			
	(b) $Al_2(SO_4)$						
	(c) K-S No. 201			"			
	(d) K-S No. 210	20 lb/in^{2}g		"			
	(e) K-S No. 531**	2 lb/in^{2}g		"			
	(f) ABCOR HFM	40 lb/in^{2}g	149.4	96.4	74.5		
(6)	(a) K-S No. 201			Comment Statement			
	(b) $Al_2(SO_4)_3$						
	(c) K-S No. 531,	40 lb/in^{2}g		106.1			
	(d) ABCOR HFM	40 lb/in^{2}g	75.6	42.4	32.2	25.8	
(7)	(a) K-S No. 201			Comment Statement			
	(b) $Al_2(SO_4)_3$						
	(c) K-S No. 211**			"			
	(d) K-S No. 210			"			
	(e) K-S No. 531			"			
	(f) ABCOR HFM,	40 lb/in^{2}g	80.2	45.6	32.6	27.7	

* Signifies a Comment Statement.
** Filter Cloth from Komline-Sanderson Corp.

K-S No.	Weave	Porosity	Material
201	Crow-Foot	36.9	Dacron
210	Crow-Foot	½ ~ 1	Dacron
211	Chain	10 ~ 15	Dacron
531	Twill	20	Nylon

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